Aptamer Selection Express: A Rapid Single-Step Selection of Double-stranded DNA Capture Elements (Briefing Charts)

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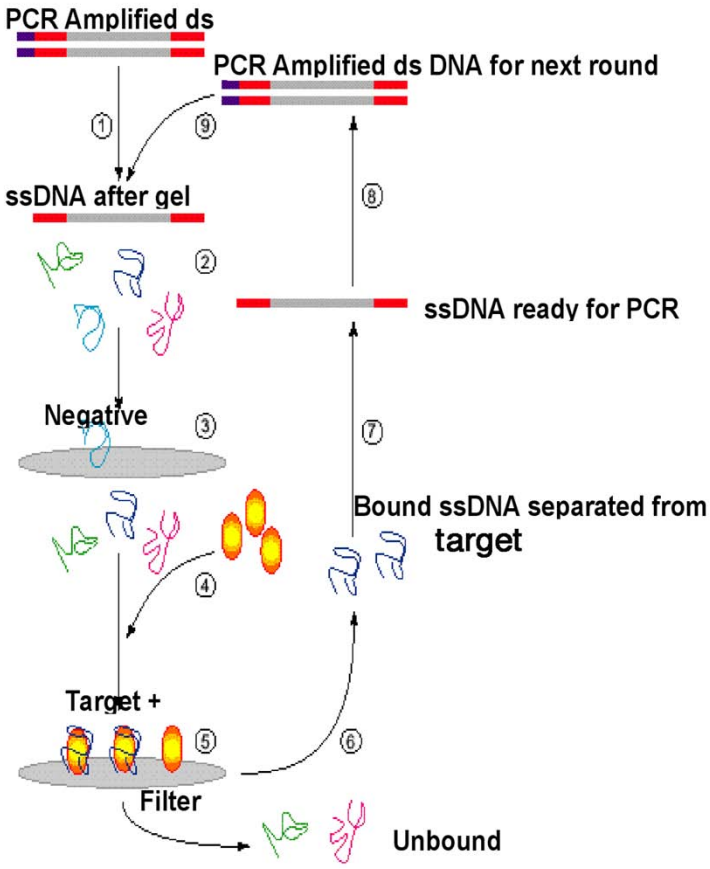
Outline

- Advantages of aptamers
- ALISA, Where we came from
- Dot Blot Format, Other possibilities
- One step Quantum Dot De-quenching Assay
  - Why we need a double-stranded DNA aptamer
- Comparing **SELEX** to Aptamer Selection Express (**ASExpP**)
- Reagentless electronic sensors (RFIDs)
- Emerging disease agents and finding an unknown
  - Why we need a rapid technique for aptamer selection
- Summary

Advantages of Aptamers

- Aptamers are smaller than antibodies – ranging from 30 to 50 nucleotides
- Do not require either animals or tissue culture for production
- Can be synthesized chemically or by PCR
- Due to the nature of DNA, they are stable in harsh environments and do not require special storage conditions
- Offer additional chemistries and modalities for further stabilization (nuclease resistance) and assays

SELEX: Selection of Aptamers



Selection begins with a library of ~10^{15} single strands of DNA. The target is bound to a filter, and a portion of the library binds to the target. The bound strands (+) are eluted from the target by heat and amplified using PCR, with the primer for the negative strand containing biotin at its 5' end. After amplification, the DNA is denatured and the (-) strands separated from the (+) strands by passing the DNA over a streptavidin column, which retains the biotin containing (-) strands. Another round of selection is begun.

Where we came from: Tularemia in Houston: PCR and Immunoassays are not the last word

Berger, "Suspicious bacteria detected: Security monitors spot germ; terrorism discounted," *The Houston (TX) Chronicle* 10 October 2003:A27

***Francisella tularensis* also discovered on Washington (DC) National Mall 24-25 Sept 2005; not reported until 1 Oct 2005**

Laboratory Investigation (2006) 1–9

www.laboratoryinvestigation.org

Technical Report

Anti-*Francisella tularensis* DNA aptamers detect tularemia antigen from different subspecies by Aptamer-Linked Immobilized Sorbent Assay

Jeevalatha Vivekananda and Johnathan L Kiel

Air Force Research Laboratory, HEPC, Brooks City-Base, TX, USA

Aptamers are powerful candidates for molecular detection of targets due to their unique recognition properties. These affinity probes can be used to recognize and bind to their targets in the various types of assays that are currently used to detect and capture molecules of interest. They are short single-stranded (ss) oligonucleotides composed of DNA or RNA sequences that are selected *in vitro* based on their affinity and specificity for the target. Using combinatorial oligonucleotide libraries, we have selected ssDNA aptamers that bind to *Francisella tularensis* subspecies (subsp) *japonica* bacterial antigen. *F. tularensis* is an intracellular, nonmotile, nonsporulating, Gram-negative bacterial pathogen that causes tularemia in man and animals. Just as antibodies have been used to detect specific targets in varying formats, it is possible that nucleic acid-binding species or aptamers could be used to specifically detect biomolecules. Aptamers offer advantages over antibody-based affinity molecules in production, regeneration and stability due to their unique chemical properties. We have successfully isolated a set of 25 unique DNA sequences that specifically bind to *F. tularensis* subspecies *japonica*. When tested in a sandwich Aptamer-Linked Immobilized Sorbent Assay (ALISA) and dot blot analysis, the aptamer cocktail exhibited specificity in its ability to bind only to tularemia bacterial antigen from subspecies *japonica, holarctica* (also known as *palaearctica*) and *tularensis* but not to *Bartonella henselae*. Moreover, there is no binding observed either to pure chicken albumin or chicken lysozyme. Thus, it appears that this novel antitularemia aptamer cocktail may find application as a detection reagent for a potential biological warfare agent like *F. tularensis*.
Laboratory Investigation advance online publication, 20 March 2006; doi:10.1038/labinvest.3700417

Keywords: DNA aptamers; *Francisella tularensis*; SELEX; ALISA; ELISA; dot blot; bioterrorism

Aptamers are single-stranded oligonucleotides with a length of tens of nucleotides, obtained by systemic evolution of ligands by exponential enrichment (SELEX) technology, exhibiting high affinity and specificity towards any given target molecule.[1,2] These single-stranded (ss) nucleic acid molecules have highly defined tertiary structures, which allow them to form stable and specific complexes with a range of different targets including small molecules such as amino acids to highly complex proteins and whole viruses.[3–7] For example, DNA-binding species have been selected that can interact with thrombin[8] and RNA aptamers have been selected that recognize a variety of cytokines.[9] These specialized molecules are analogs to antibodies in specificity and affinity with an apparent advantage of being reproduced by chemical synthesis and more easily labeled with fluorescent or other reporters during their synthesis. Comparisons of various ligand-binding aptamers with proteins that bind to their targets have shown that both nucleic acids and proteins use similar strategies for the formation of well-defined binding patterns.[10,11] Structural studies with aptamer-target complexes have demonstrated insights into molecular diversity associated with nucleic acid architecture and molecular recognition.[12] Aptamers frequently form complexes that have dissociation constants in the nanomolar range and can clearly distinguish between even closely related protein targets.[13,14] The degree of molecular distinction achieved by aptamers may surpass that of antibodies with a remarkable diversity in structure and function.[15]

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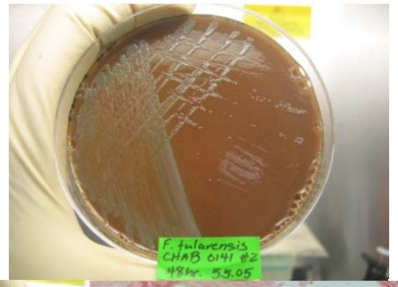
Received 9 September 2005; revised and accepted 10 February 2006; published online 20 March 2006

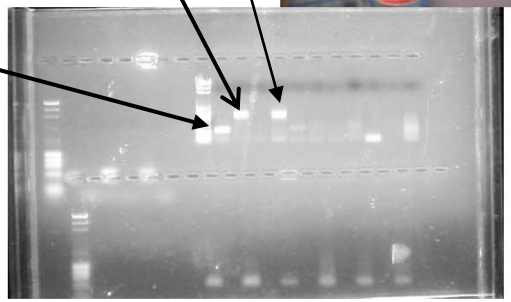
Current Methods for Tularemia Diagnosis

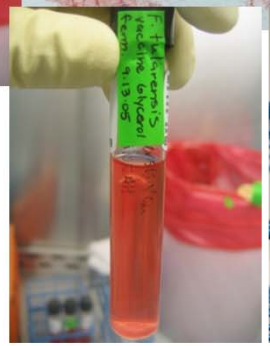
- Culture in cysteine enriched medium with glycerol (dangerous for lab personnel)
  - 1-10 Organisms can cause infection
  - Type A (most pathogenic)--glycerol catabolism positive (exceptions)
  - Type B (self-limiting)--glycerol catabolism negative
- CDC PCR method
  - Smaller product--Type A
  - Larger product (insertion)--Type B
- Immunoassays (ELISA and Agglutination)
  - For antibodies to F. tularensis in serum
  - Can be adapted to find bacterial antigen
  - Not type specific

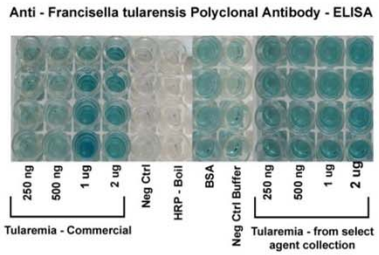
The Houston cat after preliminary culture

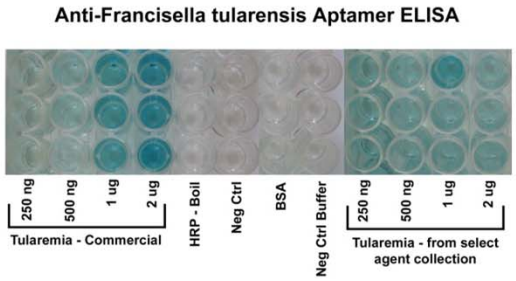
Type B

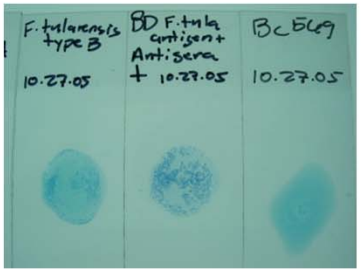




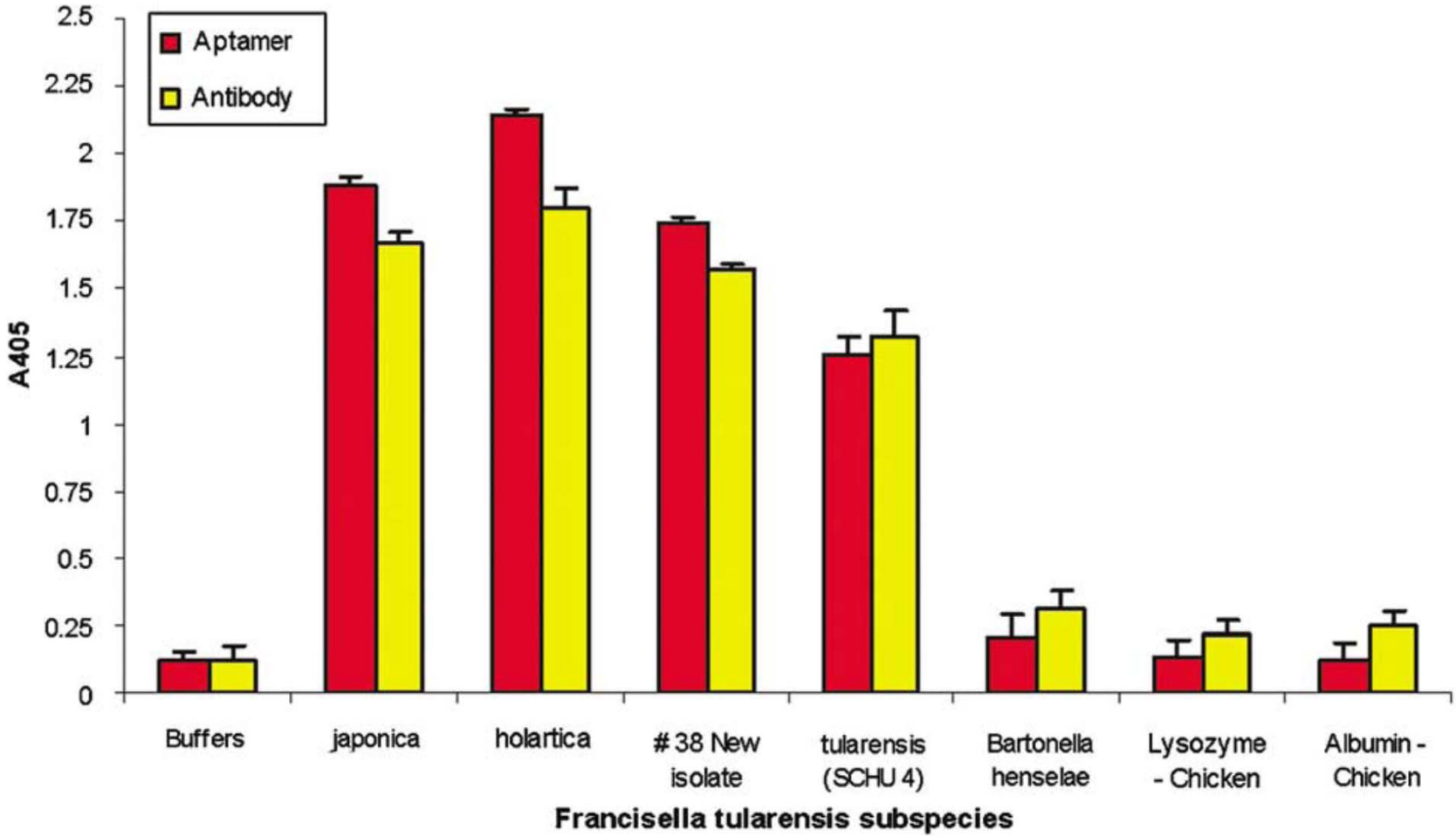








Tularemia in Houston:
PCR is not always the last word



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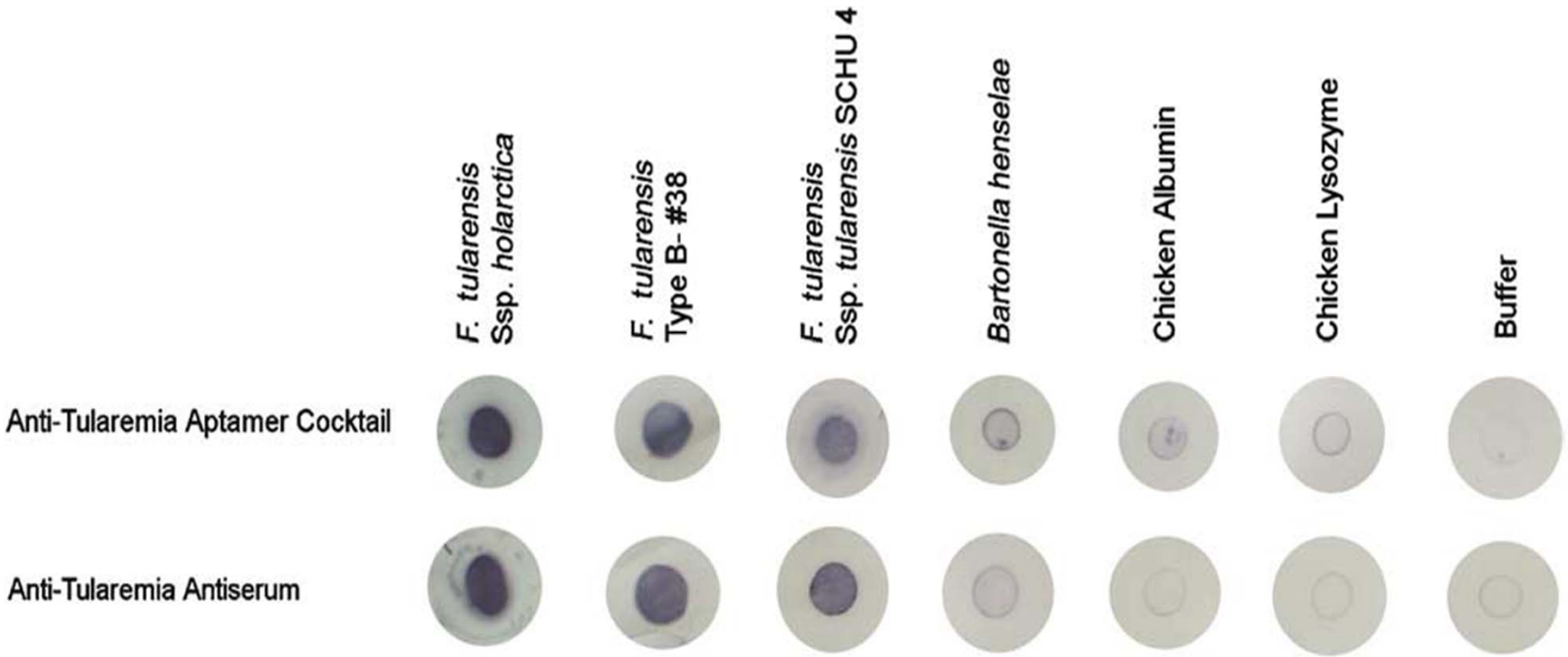


Sensitivity of Aptamers for Detecting *Bacillus thuringiensis* Spores and *Francisella tularensis*

J Fluoresc (2007) 17:193–199
DOI 10.1007/s10895-007-0158-4

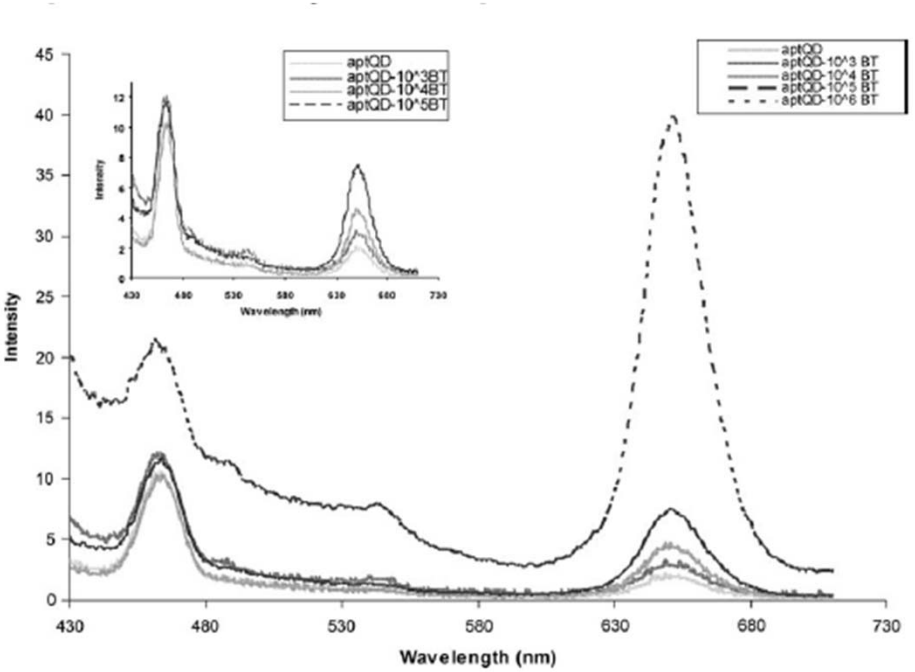


Fig. 3 Fluorescence Spectra of aptamer-QD bound to BT spores. Dashed line is the spectrum of aptamer-QD reacted with 10^6 CFU of BT (apt QD-10^6-BT). Other dilutions are shown in the legend area. An inset in the upper left hand corner represents the same data with the exclusion of the data for 10^6 CFU of aptamer-QD bound to BT spores

Laboratory Investigation advance online publication, 20 March 2006; doi:10.1038/labinvest.3700417

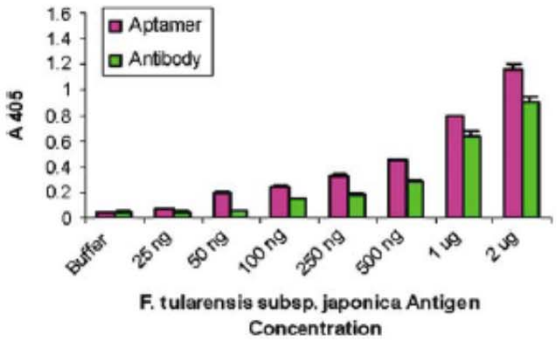


Figure 1 Sensitivity of anti-tularemia aptamer cocktail for *F. tularensis* subspecies *japonica* antigen and anti-tularemia anti-serum as assessed by ALISA and ELISA. The assays were performed as described in 'Materials and methods'. The data are presented as OD at 405 nm *vs* antigen quantity. Averages of four replication measurements are shown in the figure.

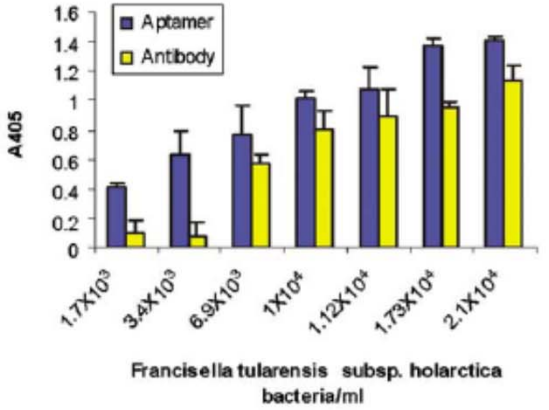
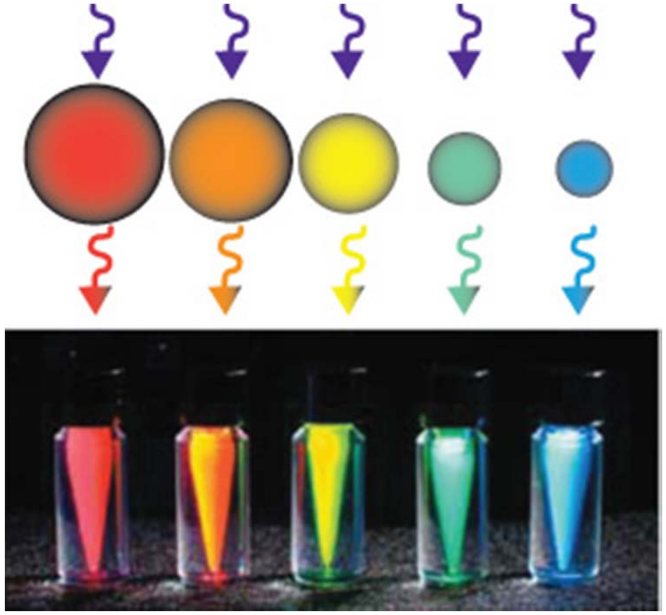
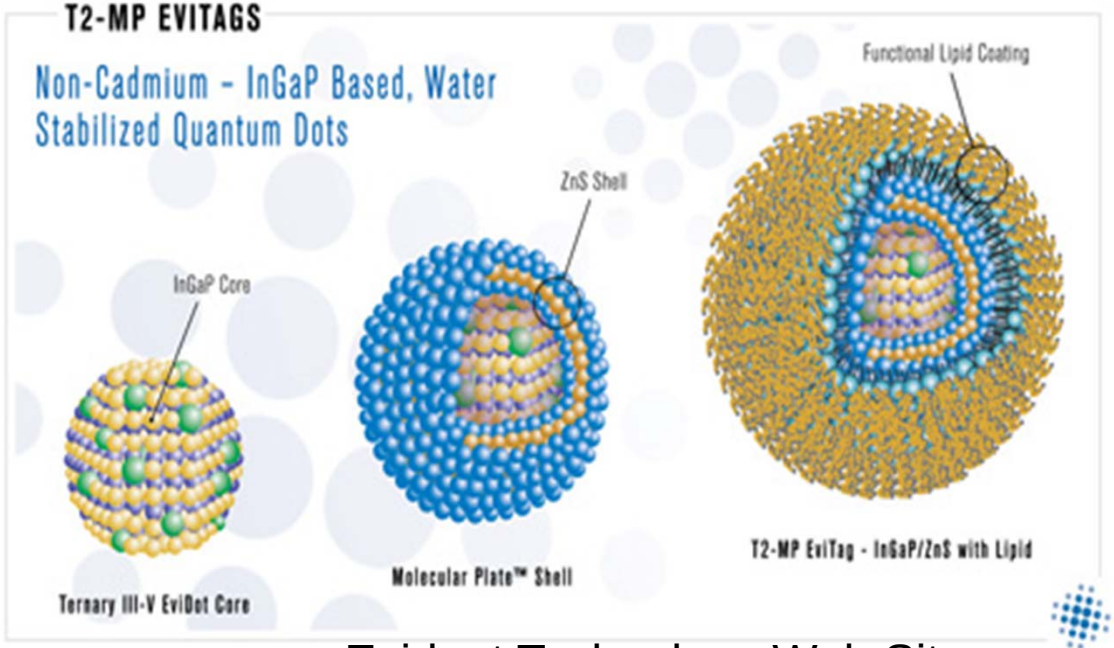


Figure 2 Tularemia bacterial antigen binding to anti-tularemia aptamer cocktail and anti-tularemia polyclonal antibodies as assessed by ALISA and ELISA using HRP activity. The assays were performed as described in 'Materials and methods'. The bacterial antigen used in the binding assay was prepared from *F. tularensis* subspecies *holarctica* (live vaccine strain). The data are plotted as OD at 405 nm *vs* number of bacteria/ml. Averages of triplicate measurements are shown in the figure.

Quantum Dots

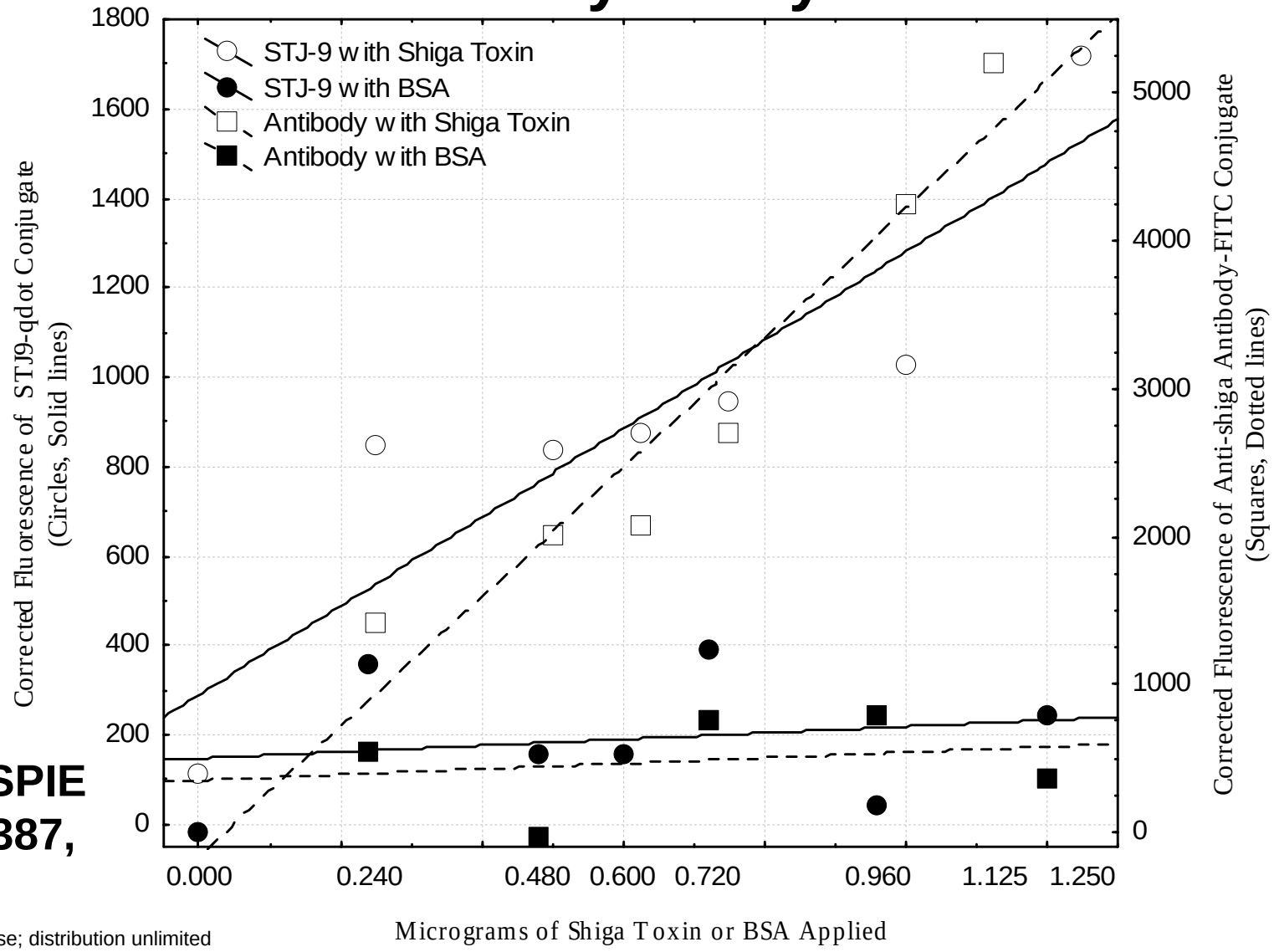
- Quantum Dots
  - Very bright
  - Resistant to photo-bleaching
  - One excitation wave length
  - Vendors
    - Evident Technologies: T1(block co-polymer) and T2 (lipid)
    - Invitrogen (polymer)





Evident Technology Web Site

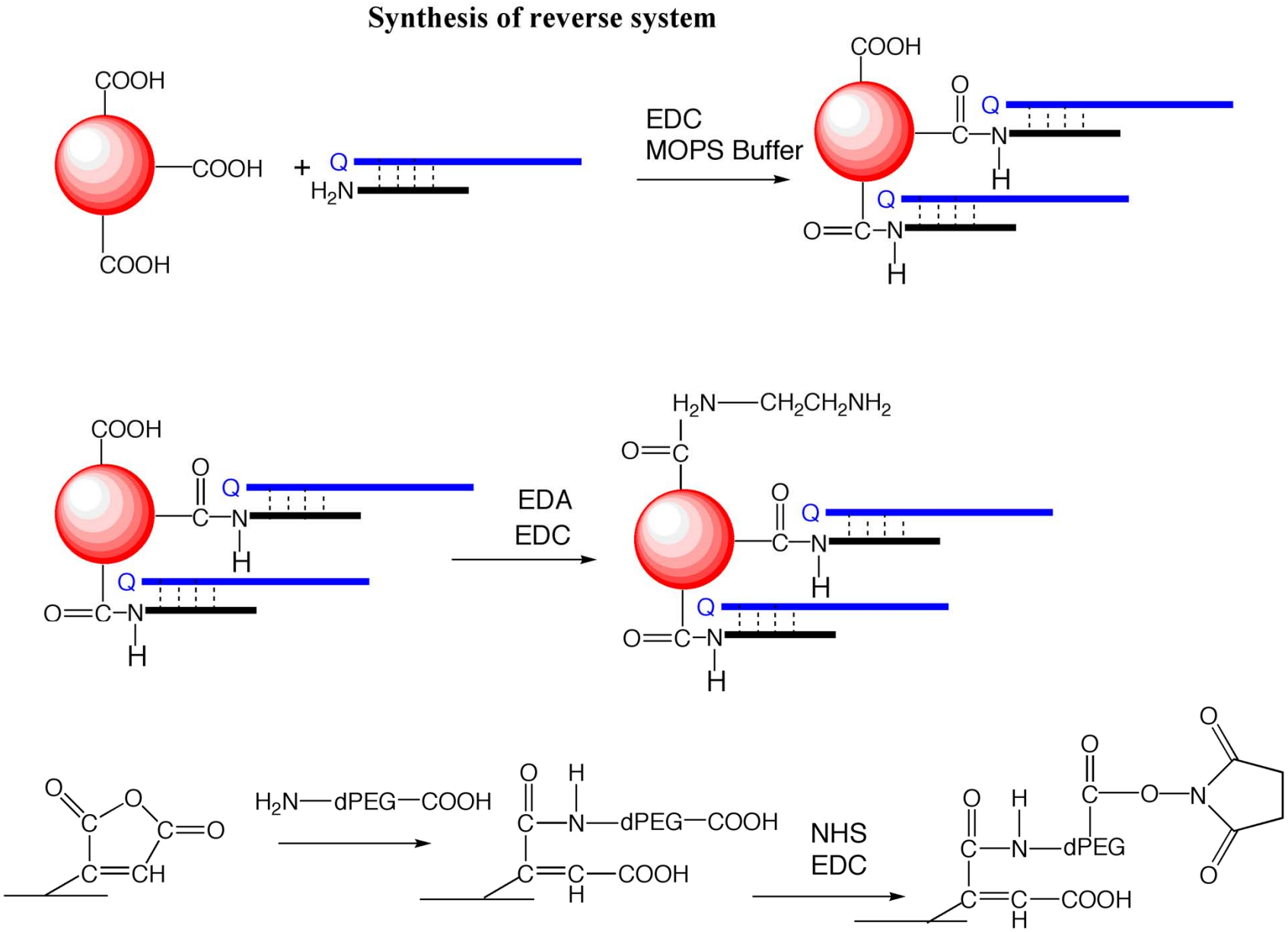
ALISA approach: Quantum Dot DCE Assay for Shiga Toxin Compared to FITC Antibody Assay



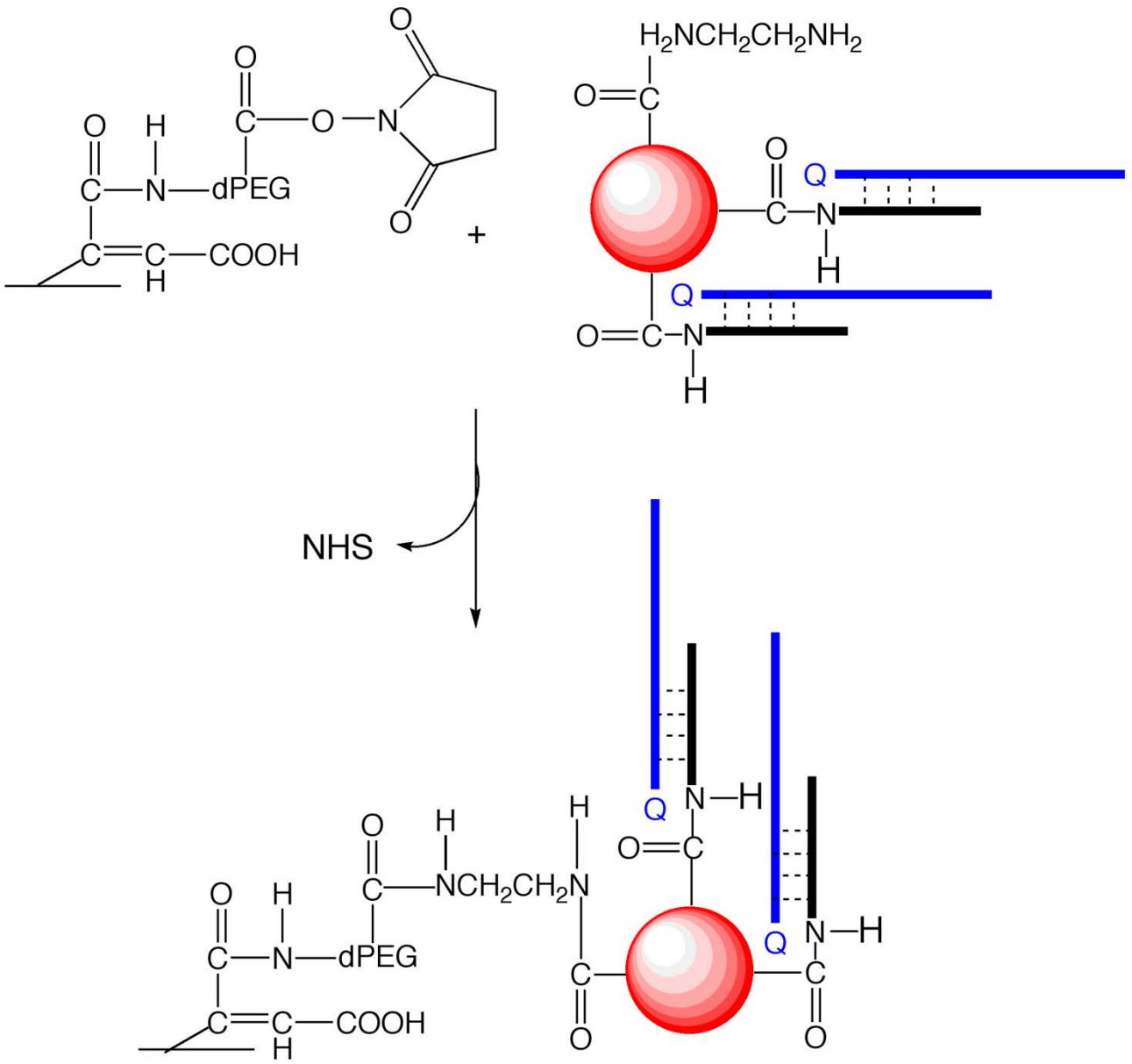
DCE=DNA Capture Element aptamer

(Kiel et al, SPIE 5617: 382-387, 2004)

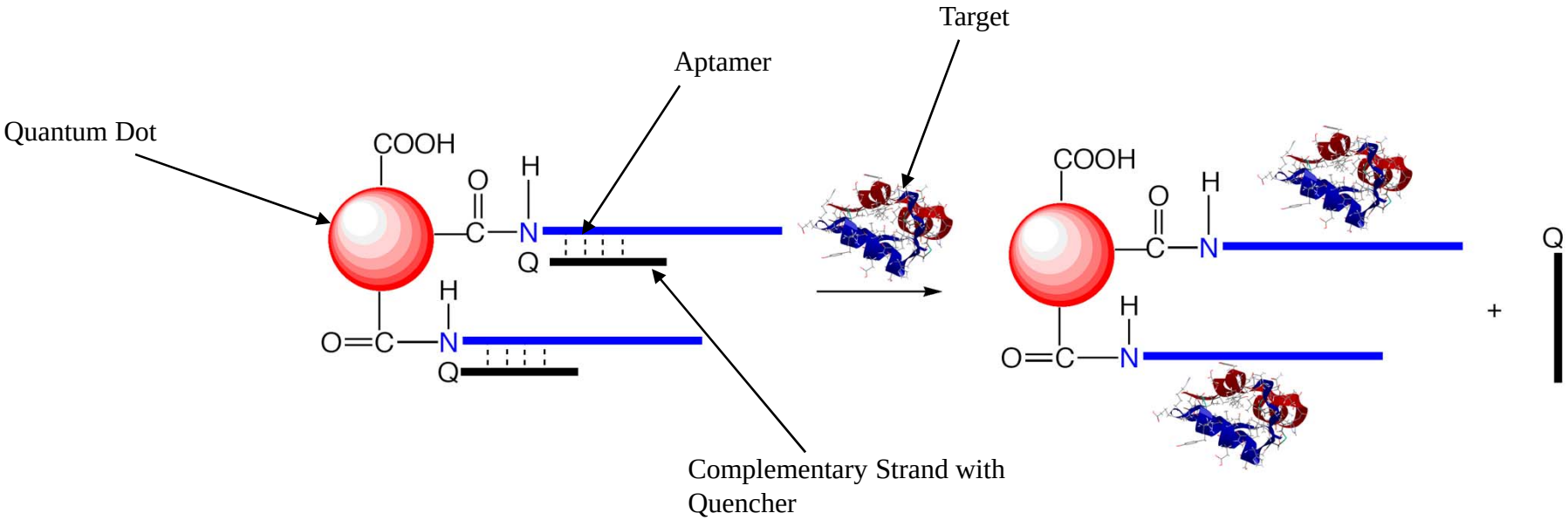
Immobilization of Aptamer/Qdot

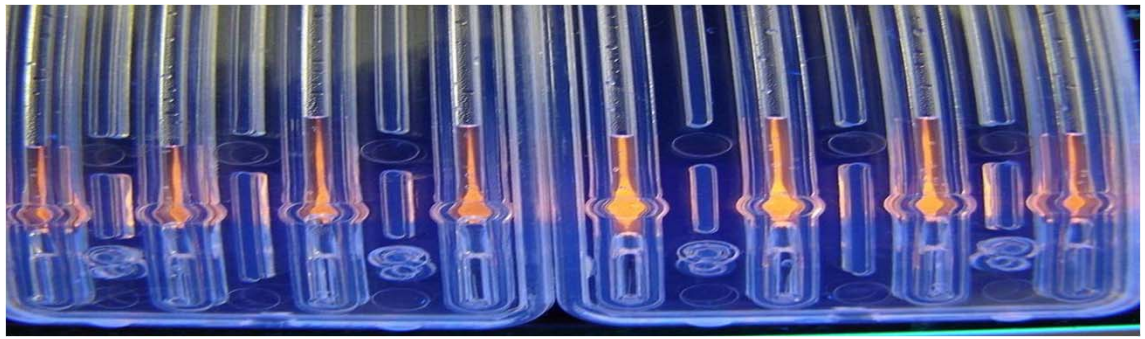


Immobilization of Aptamer/Qdot



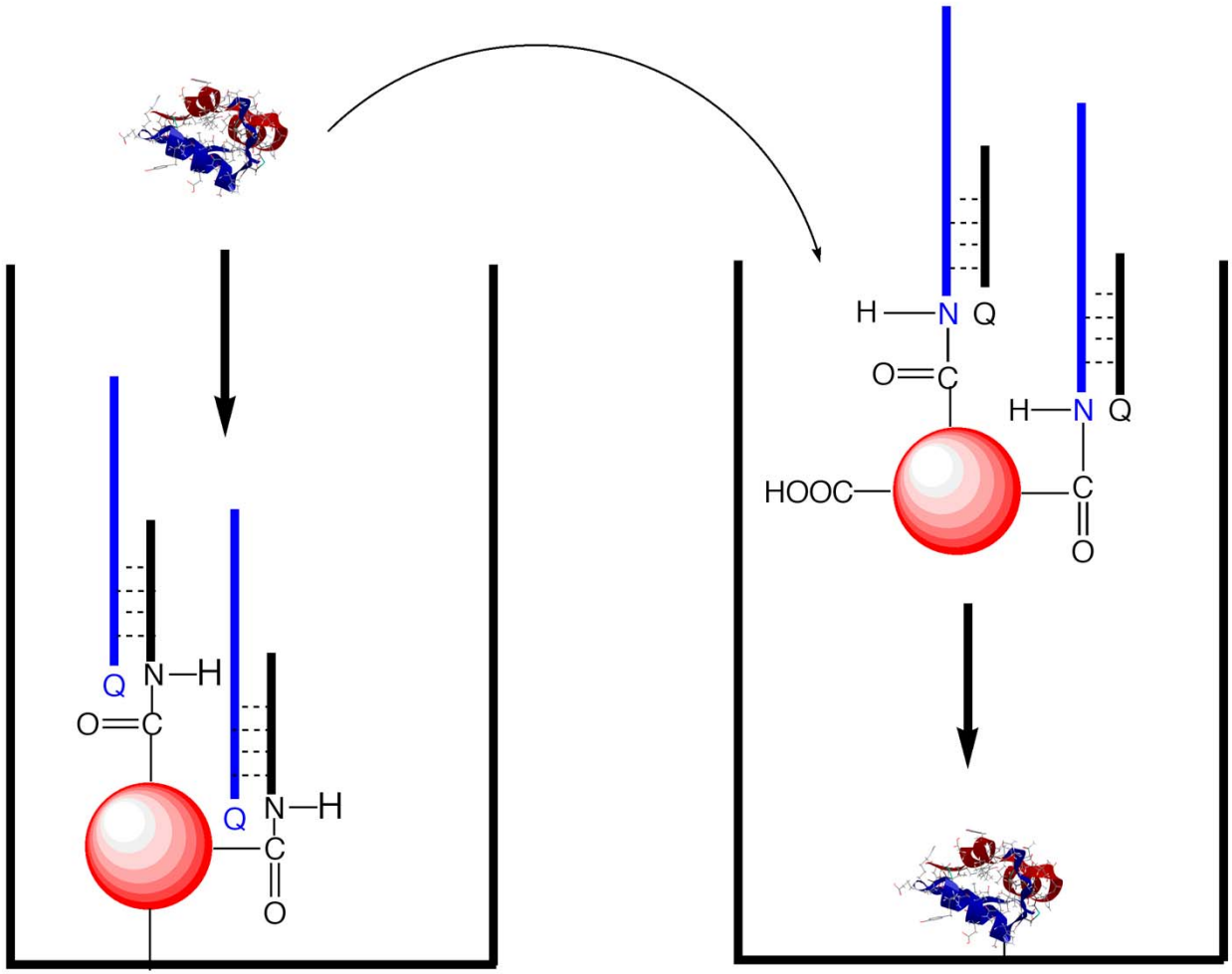
Quenching/Dequenching





Quenched Dequenched

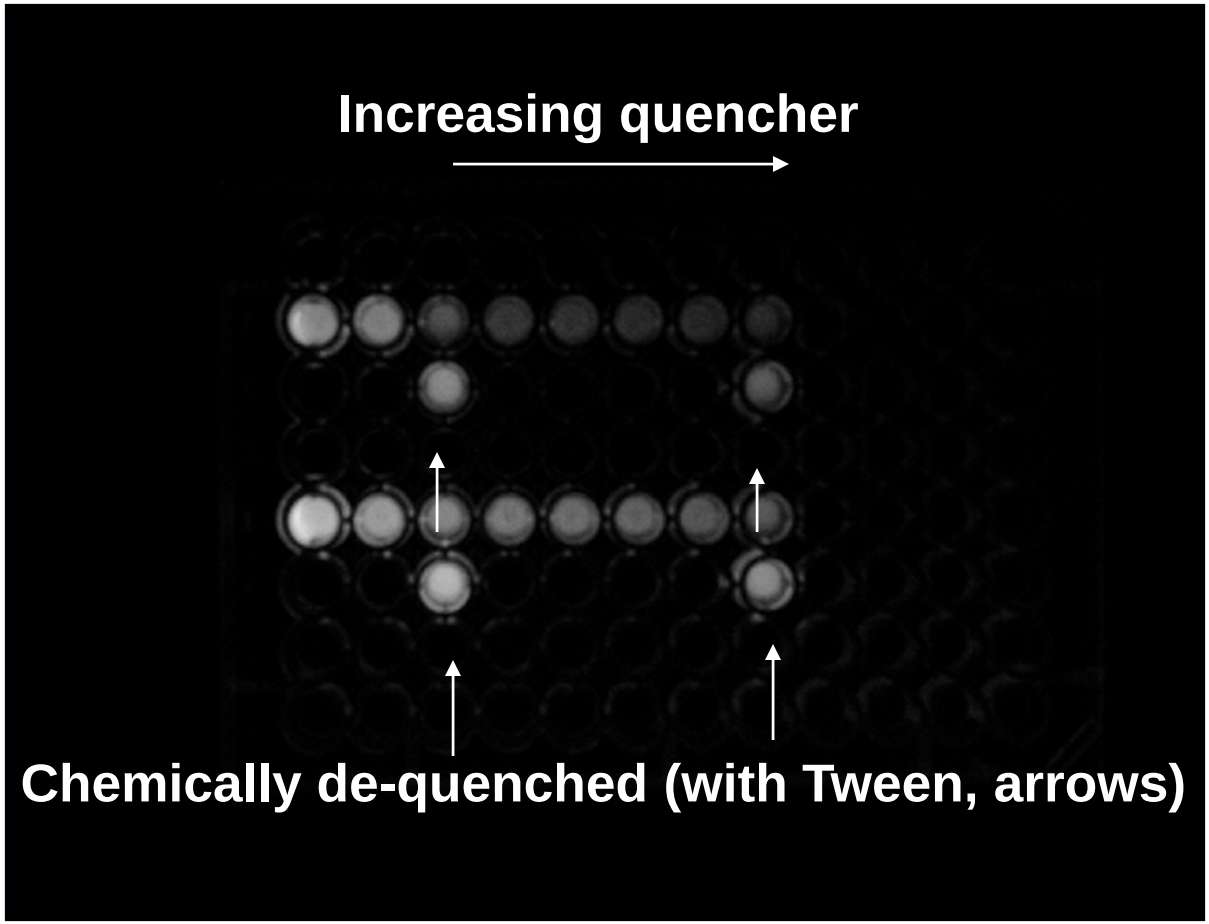
Possible type of Assays



ELISA-like Assay

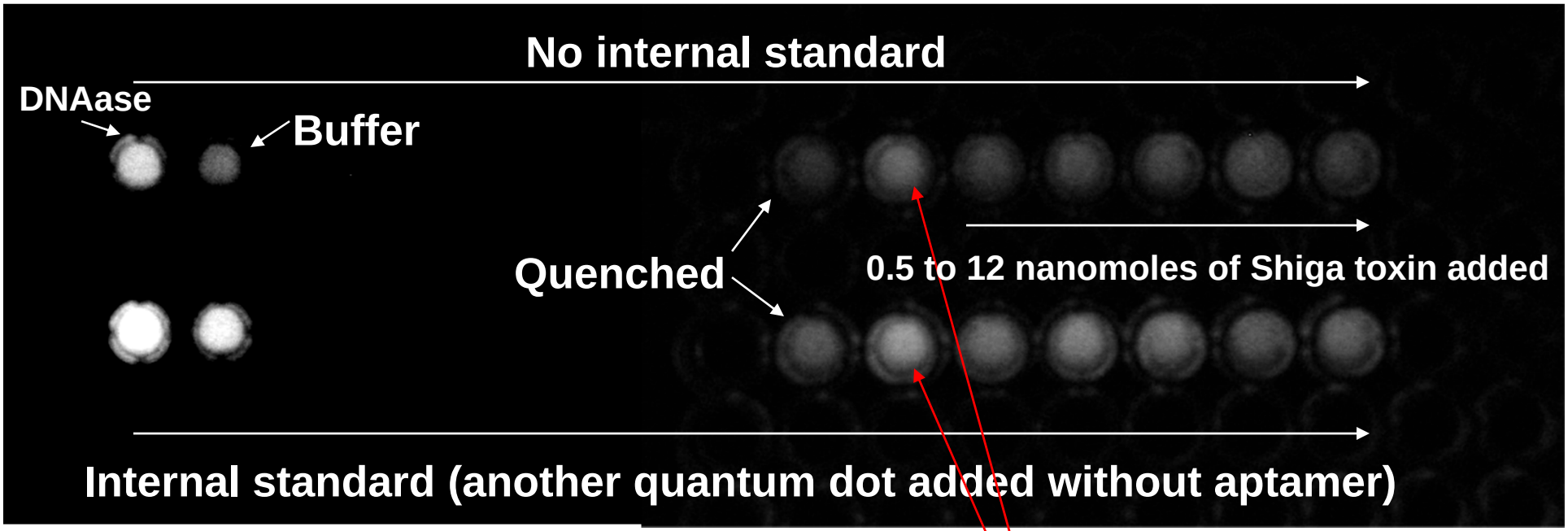
Competitive type Assay

Microtiter Demonstration (Macro Visualization) of De-quenching of Quantum Dots (Positive Control)



Dr Eric Holwitt and Ms Veronica (Franz) Sorola

Microtiter Demonstration (Macro Visualization) of De-quenching of Quantum Dots Specific for Shiga Toxin with Shiga Toxin



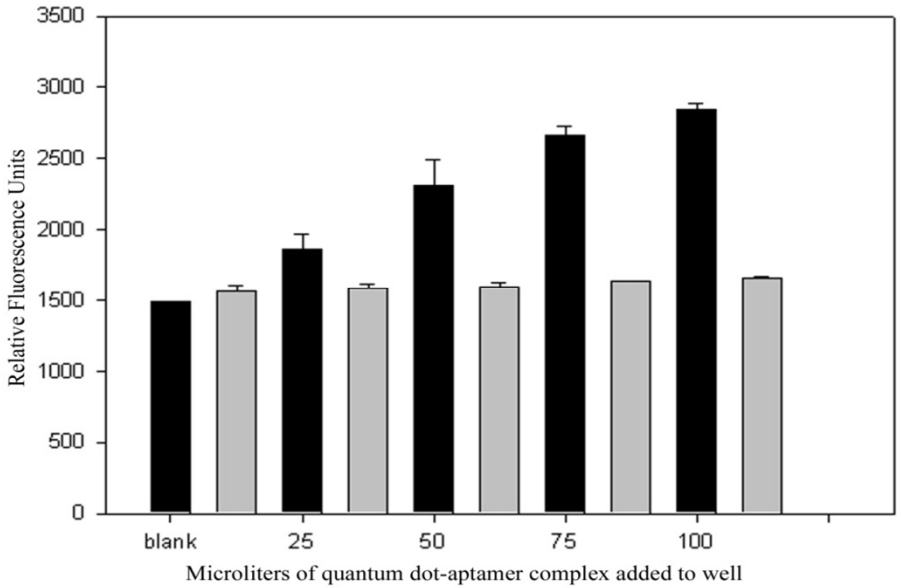
Dr Eric Holwitt and Ms Veronica Sorola

DNAase positive control

Note that the de-quenching with Shiga toxin with various amounts reached a maximum and then declined somewhat; this was a result of cross-linking of excess Shiga toxin, causing precipitation

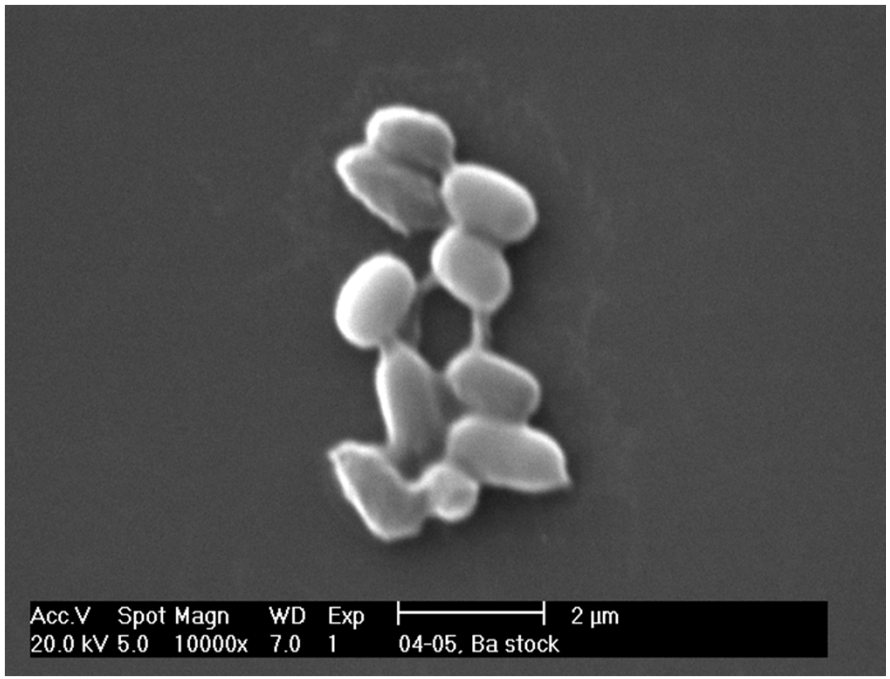
Aptamer/Quenched Quantum Dots Response to Shiga Toxin or Ovalbumin using SELEX DNA Aptamers

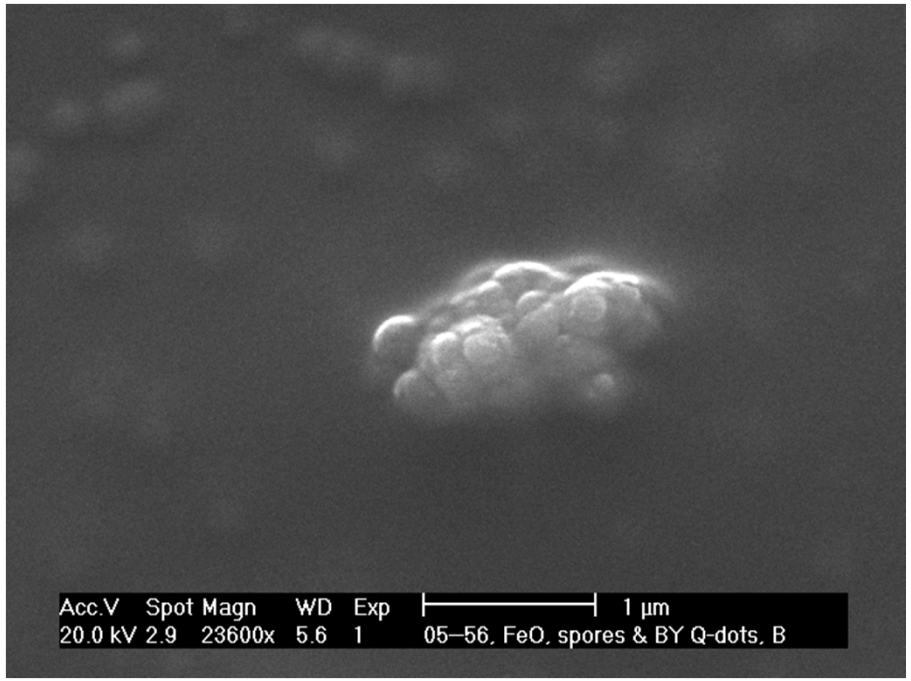
Kiel, J. L., Holwitt, E. A., and Sorola, V. K. Select Agent Recovery and Identification Using Aptamer-Linked Immobilized Sorbent Assay. Proceedings of the CB Medical Treatment Symposium, Spietz Laboratory, Switzerland **CBMTS VII** (electronic publication), 33, 1-7 (2008)

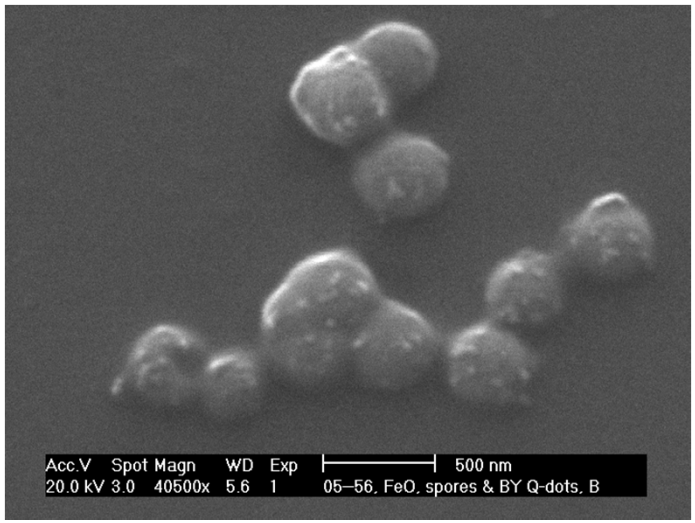


Shiga toxin: black bars
Ovalbumin: gray bars

Nanoparticles and Nanocrystals Attached to Anthrax Spores: Contact Detection/Identification and Collection

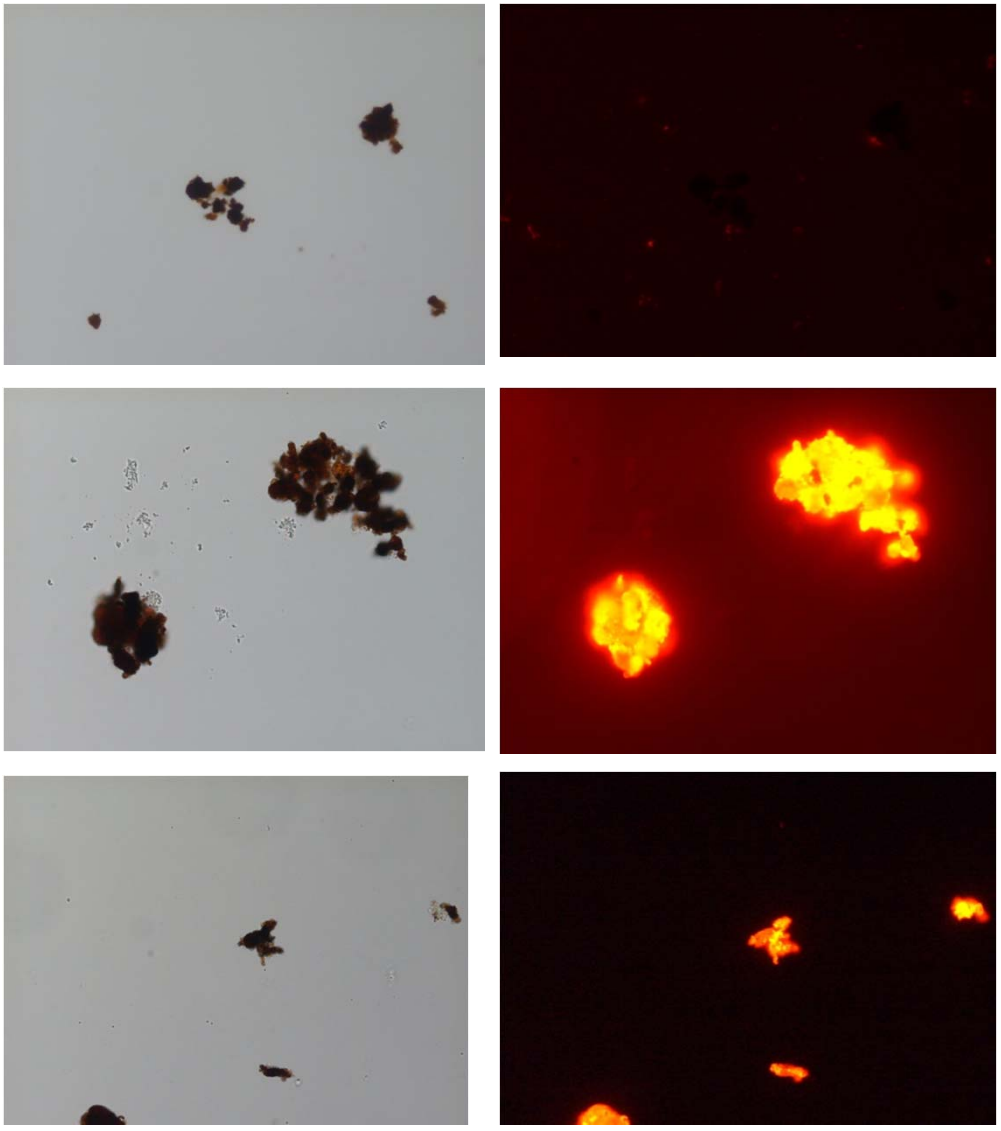






Paramagnetic Particles with DNA Capture Element (DNA Aptamers) and Quantum Dots (QD) Attached

Bright Field UV Excitation



Control: No specifically bound Dots

Anthrax spores linked to DCE, QD and paramagnetic particles

Bacillus atropheus (globigii) **spores linked to DCE, QD and paramagnetic particles for anthrax spores**

Aptamer Based Agent Detection

GOAL: Man portable detection of biological agents in the field

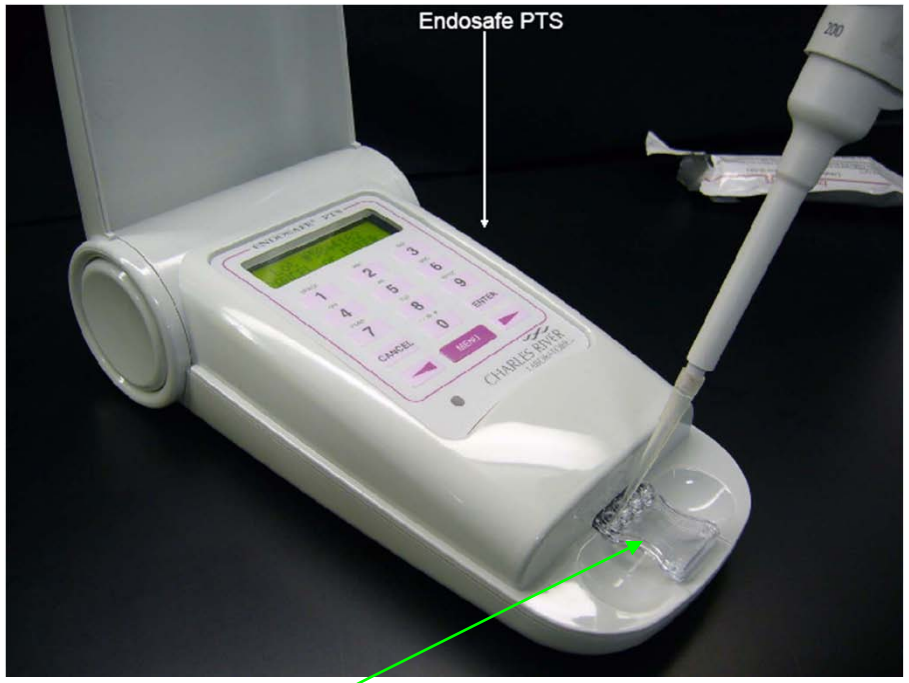
The Portable Test Laboratory has been flown on the International Space Station

Identifies positive sample in field allowing for further analysis in a controlled location !

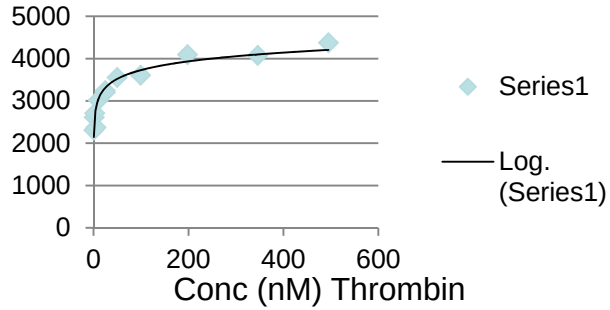
The Portable Test Laboratory has been tested in conditions of extreme heat and cold

AFRL/CRL Device

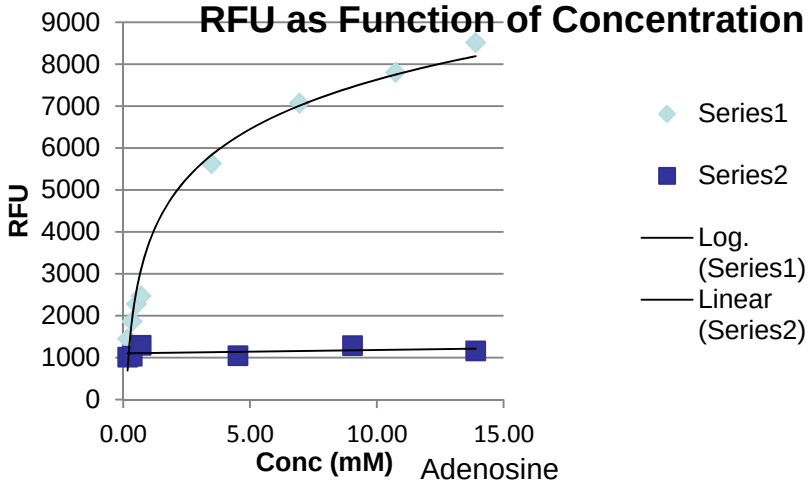
Currently Marketed Portable Test Lab



Cassette loaded into Portable Test Laboratory

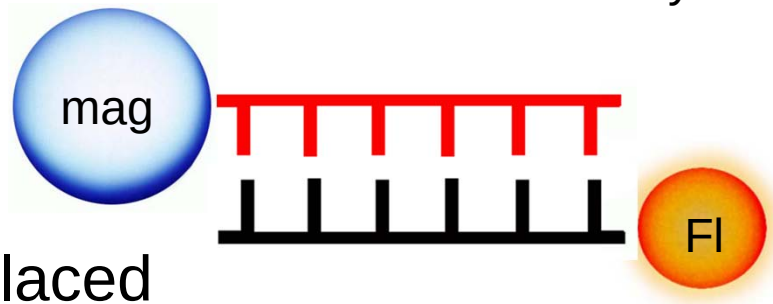


- Cartridge provides ability to retrieve samples for identification and analysis of substance
- Device tests and identifies the contained specimen
- Current system measures fluorescence
- 9.25x4.625x2.50 inches
- Battery Operation for 4 hrs
- ~ 2 lbs

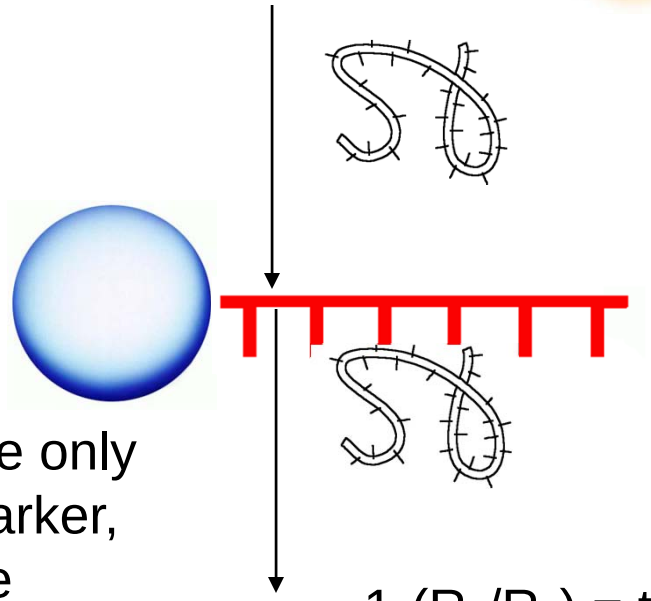


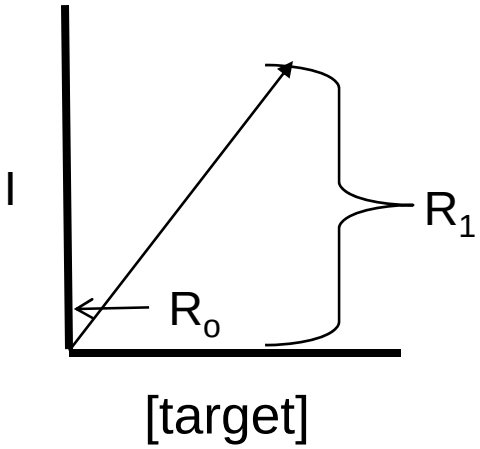
Magnetic Nanoparticle or Microparticle and Quantum Dot Separation

Internal Standard Subtractive Ratio Assay Method



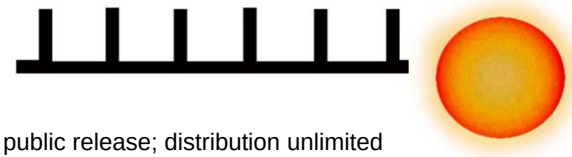
Quantum Dot is displaced by Target: Increase in fluorescence in downstream optical window



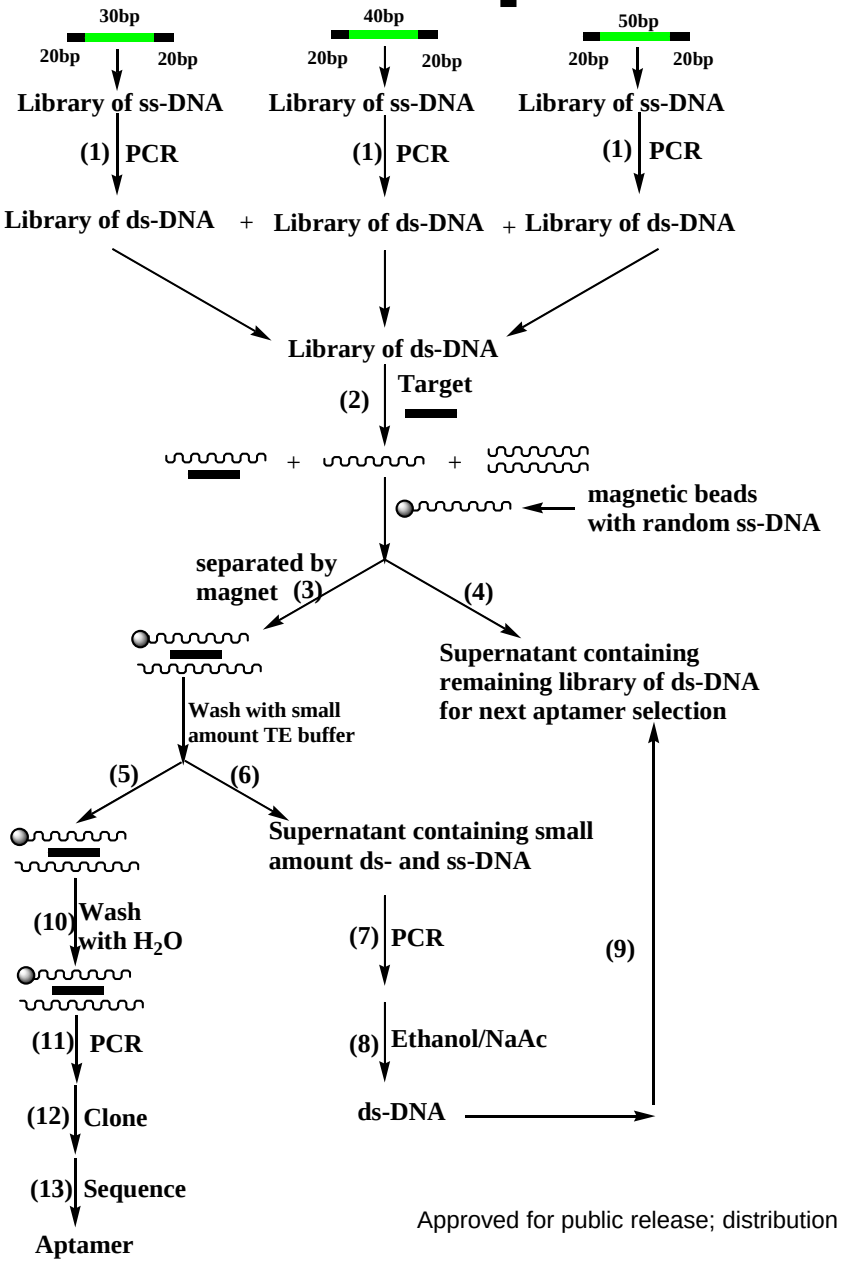


Target can be specific to live only marker, dead and/or live marker, and metallic marker in three separate channels of microfluidics cassette

$1-(R_0/R_1)$ = target to sensor ratio



ASExpP



Bot Tox Aptamers: **SELEX** and **ASExpP**

Selected by ASExpP against BoTox, type A-light chain (for DCE-1)
1(+). AgTCTAgAgggCCCCAgAAT**ACACCCgACAACTAgAT ACCCATCAAAAgT**CCAgCAAAggATgCAggggT 1(-). ACCCCTgCATCCTTTgCTgg**ACTTTTgATgggTATCTA gTTgTCgggTgT**ATTCTggggCCCTCTAgACT
Selected by ASExpP against BoTox, type B-light chain (for DCE-2)
2(+). AgTCTAgAgggCCCCAgAAT**TATCCACTAgCgggAAgT AgTACATCTCAC**CCAgCAAAggATgCAggggT
2(-). ACCCCTgCATCCTTTgCTgg**gTgAgATgTACTACTTCC CgCTAgTggATA**ATTCTggggCCCTCTAgACT
Selected by SELEX against BoTox, type A-light chain (for DCE-3)
3(+). CATCCgTCACACCTgCTCTg**gggATgTgTggTgTTggCT CCCgTATCAAgggCgAATTCT**
3(-). gTAggCAgTgTggACgAgACC**CCTACACACCACAACC gAgggCATAgTTCCCgCTTAAgA**
Selected by SELEX against BoTox Holotoxin (for DCE -4)
4(+). CATCCgTCACACCTgCTCTg**CTATCACATgCCTgCTg AAgTggTgTTggCTCCCgTATCA**
4(-). gTAggCAgTgTggACgAgAC**gATAgTgTACggACgACTTC ACCACAACCgAgggCATAgT**

Double-Strand DNA Response De-quenching Using ASExpP vs. SELEX Aptamers against Bot Tox A

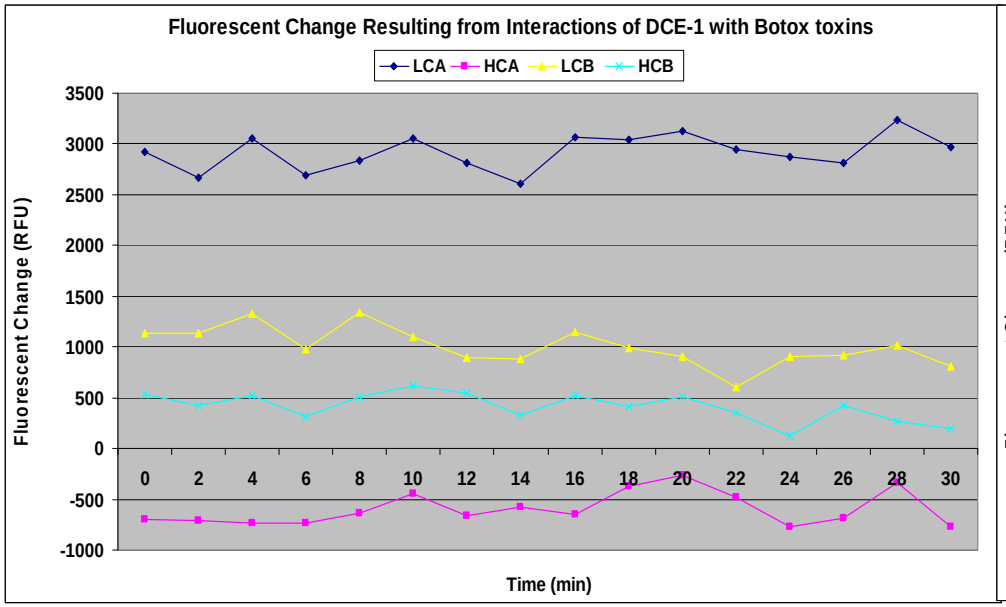


Figure A. Fluorescence change resulting from the interactions of DCE-1 (made from aptamer against BoTox, type A-light chain by **ASExpP process**) with different types of BoTox.

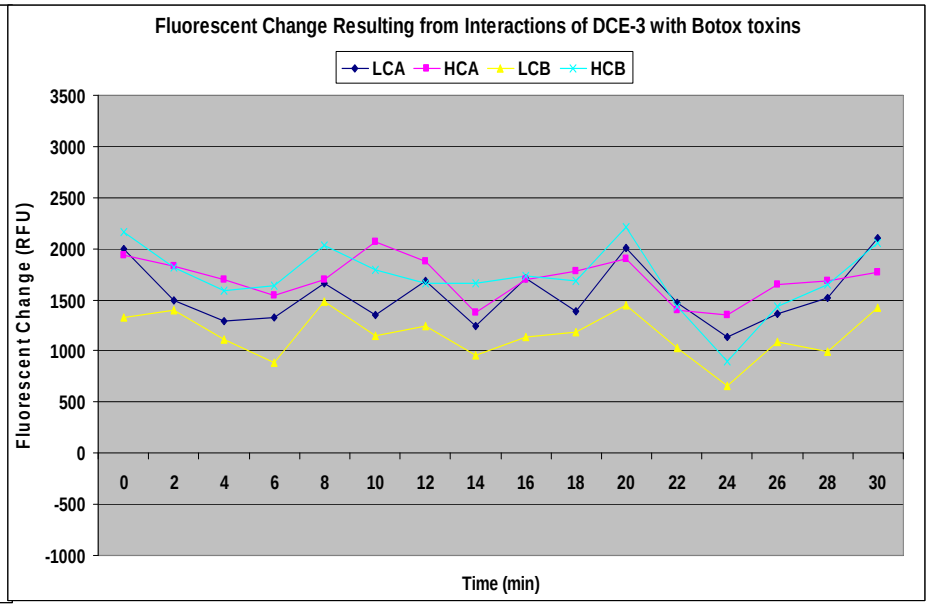
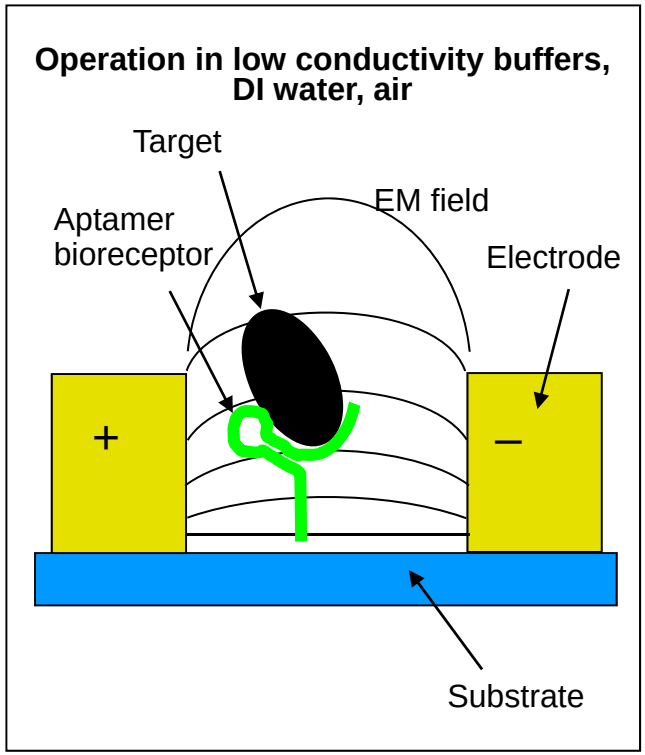


Figure B. Fluorescence change resulting from the interactions of DCE-3 (made from aptamer against BoTox, type A-light chain by **SELEX process**) with different types of BoTox.

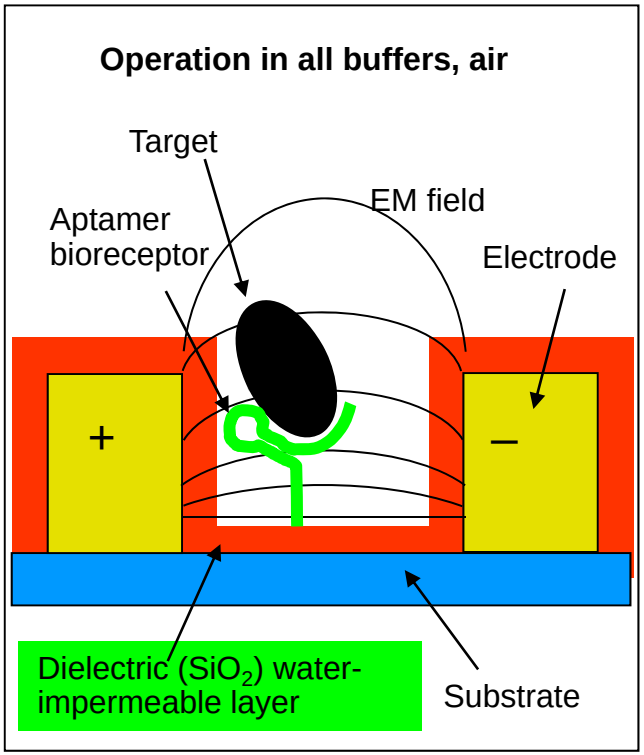
•Fan,M., McBurnett, S. R., Andrews, C. J., Allman, A. M., Bruno, J. G., and Kiel, J. L.. Aptamer Selection Express: A Novel Method for Rapid Single-Step Selection and Sensing of Aptamers. J Biomol Tech **19**(5), 311–319 (December 2008).

GE GRC Concepts: Complementary Sensing Structures

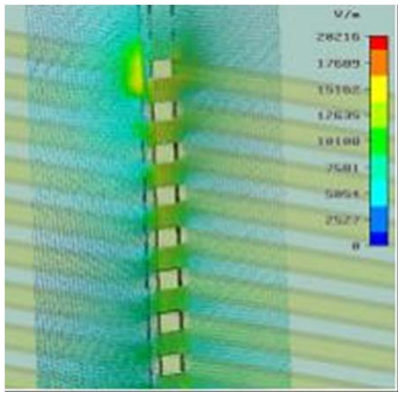
Bare electrode structures



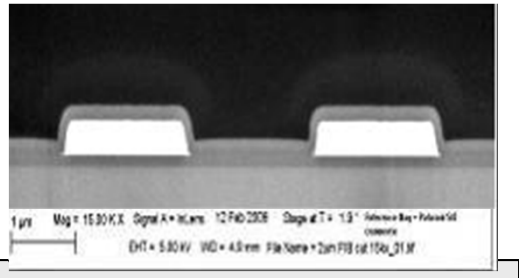
SiO_2-coated electrode structures



3D model of EM field



Nanofabricated sensor

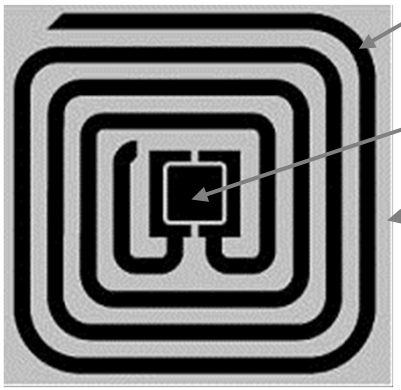


Detection of changes in capacitance and resistance of sensing gap between electrodes provides improved sensitivity and stability and rejects interferences

R. A. Potyrailo, C. Surman, R. Chen, S. Go, K. Dovidenko, and W. G. Morris, General Electric Company, Global Research Center, Niskayuna, NY, USA; E. Holwitt, V. Sorola, and J. L. Kiel, Air Force Research Lab RHPC, Brooks City-Base, TX, USA. Label-Free Biosensing Using Passive Radio-Frequency Identification (RFID) Sensors.15th International Conference on Solid-State Sensors, Actuators and Microsystems - Transducers'09 , 21-25, June 2009, Denver, Colorado USA

GE GRC: Two Designs of RFID Sensing Electrodes

Full antenna sensing structure



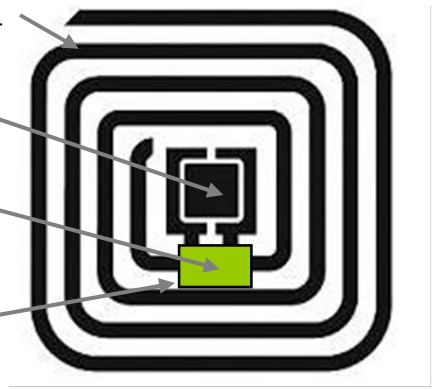
RFID sensor antenna

IC memory chip

Sensing material

Complementary sensing region

Complementary sensing structure



Pros
Simple design
Ease of fabrication

Cons
Reagent cost

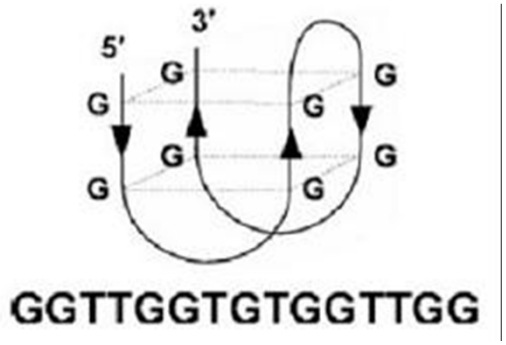
Pros
Smaller sensing area
Ease to deposit sensing films
Highest sensitivity

Cons
Medium fabrication difficulty

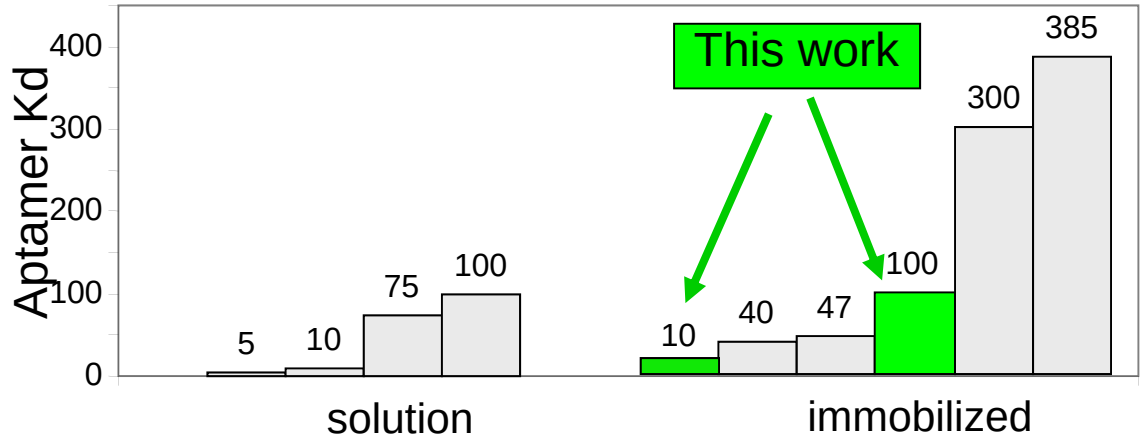
R. A. Potyrailo, C. Surman, R. Chen, S. Go, K. Dovidenko, and W. G. Morris, General Electric Company, Global Research Center, Niskayuna, NY, USA; E. Holwitt, V. Sorola, and J. L. Kiel, Air Force Research Lab RHPC, Brooks City-Base, TX, USA. Label-Free Biosensing Using Passive Radio-Frequency Identification (RFID) Sensors.15th International Conference on Solid-State Sensors, Actuators and Microsystems - Transducers'09 , 21-25, June 2009, Denver, Colorado USA

GE GRC: Analysis of K_d for Thrombin Aptamer

Reference	Aptamer use	Sensing format	K_d
Ostatna, V. et al. Anal. Bioanal. Chem. 2008, 391(5), 1861	3'-biotin or 3'-SH onto avidin, Au and dendrimer surfaces	SPR	**40-385nM**
Potyrailo, R., et al, Anal. Chem., 1998, 70, 3419.	3'-C7 glass slide immobilized	ATR-fluorescence anisotropy	**47nM**
Lee, M.; Walt, D. R., Anal. Biochem. 2000, 282, (1), 142.	5'-NH-C6 on silica beads	Competition, fluorescence	**300nM**
GE GRC (THIS WORK)	Functionalized aptamers on glass slide	Fluorescence	**10-100nM**
Li, J. J. et al. Biochem. Biophys. Res. Comm. 2002, 292, (1), 31.	Solution	Molecular beacon, fluorescence	**5.20 ± 0.49 nM**
Hamaguchi, N., et al. *Anal. Biochem.* **2001,** 294, (2), 126.	Solution	Molecular beacon, fluorescence	**10nM**
Tasset, D. M. et al. J. Mol. Biol.1997, 272, (5), 688.	Solution	Filter binding	**75-100nM**

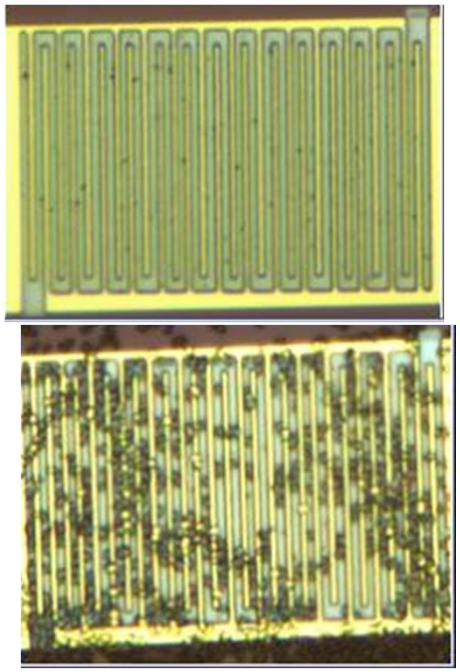


Thrombin aptamer: Bock et al., *Nature* **355**: 564-566, 1992.



R. A. Potyrailo, C. Surman, R. Chen, S. Go, K. Dovidenko, and W. G. Morris, General Electric Company, Global Research Center, Niskayuna, NY, USA; E. Holwitt, V. Sorola, and J. L. Kiel, Air Force Research Lab RHPC, Brooks City-Base, TX, USA.
Label-Free Biosensing Using Passive Radio-Frequency Identification (RFID) Sensors.15th International Conference on Solid-State Sensors, Actuators and Microsystems - Transducers'09 , 21-25, June 2009, Denver, Colorado USA

GE GRC: *Spore detection*: Characterization of 2-D Bare Nanogap FIB-fabricated Electrodes



Detection limit of BG spores = 35 spores
Most techniques except for culture (1 spore) detect a minimum of 100-100,000 spores

Potyrailo et al, 2009

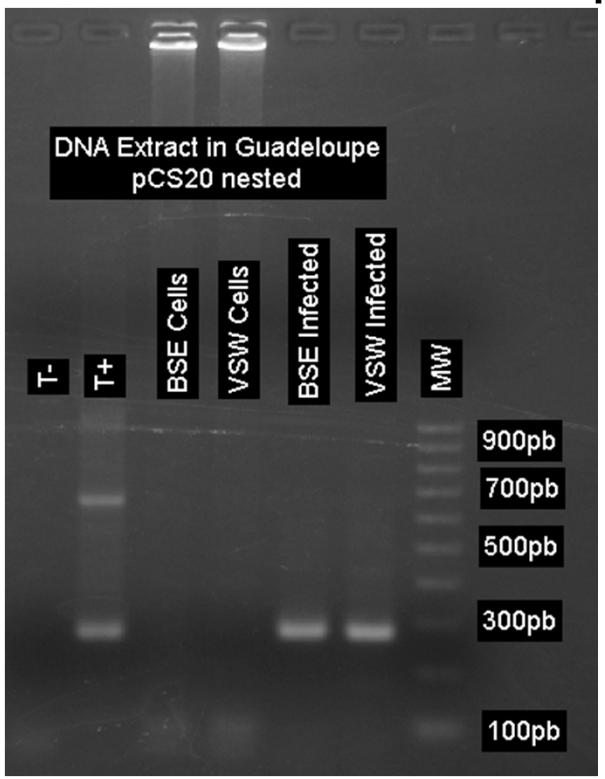
Emerging Exotic Pathogens: Heartwater and Viper Plague

Amblyomma species responsible for transmission of Ehrlichia ruminantium (Photo courtesy of APHIS-USDA)

- **Heartwater**
  - **Tick-borne disease: *Amblyomma variegatum, A. hebraeum, A. lepidum, A. maculatum*, other Amblyomma tick carriers**
  - **Causal agent: *Cowdria ruminantium*, now *Ehrlichia ruminantium***
- **Imminent threat to Western Hemisphere**
  - **Mortality in cattle and other ruminants: excess of 70%**
  - **Has been found in African spurred tortoises (*Geochelone sulcata*) and leopard tortoises (*Geochelone pardalis*)**
  - **Is now in Caribbean Islands**
    - **Antigua**
    - **Guadeloupe**
    - **Marie Galante**
    - **Perhaps Cuba**
- **Viper Plague, a mimic of heartwater, and associated ticks entered the USA in 2002**
- **VP rickettsia was isolated in viper cells and propagated in turtle cells, but also infects bovine endothelial cells, and human cells (HeLa)**

Kiel, J. L. , Alarcon, R. M., Parker, J. L., Vivekananda, J., Gonzalez, Y. B., Stribling, L. J. V., and Andrews, C., Emerging Tick-Borne Disease in African Vipers Caused by a Cowdria-Like Organism, Ann. N.Y. Acad. Sci. 1081: 434-442, 2006

Molecular Biology Confusion (Standard Diagnostic PCR) Between Heartwater and Viper Plague



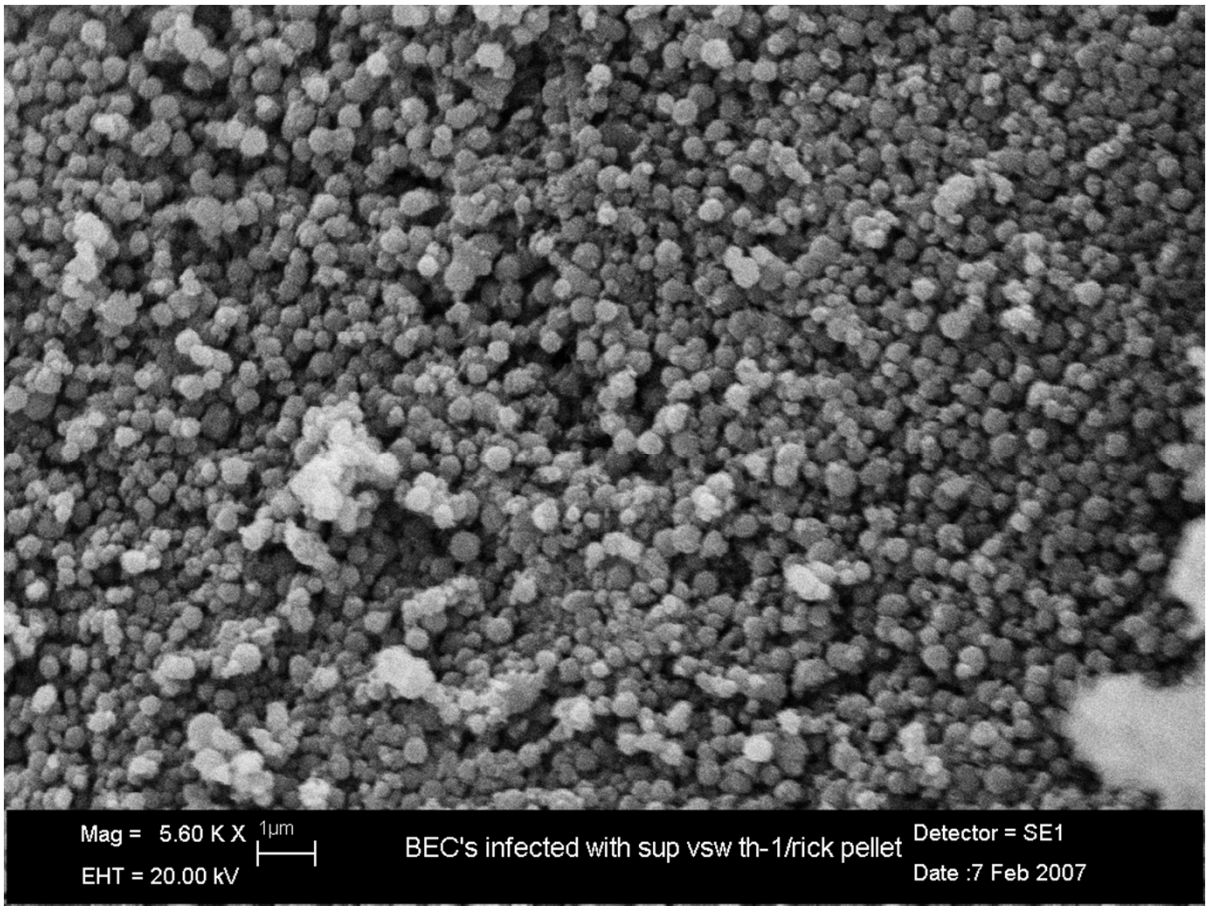
Nested PCR pCS20: AB128/129/130

Kiel, J.L., Gonzalez, Y., Parker, J.E., Andrews, C., Martinez, D., Vacheiry, N., LeFrancois, T. Viral association with the elusive rickettsia of viper plague from Ghana, West Africa. Annals of the New York Academy of Sciences 1149, 318-321 (2008).

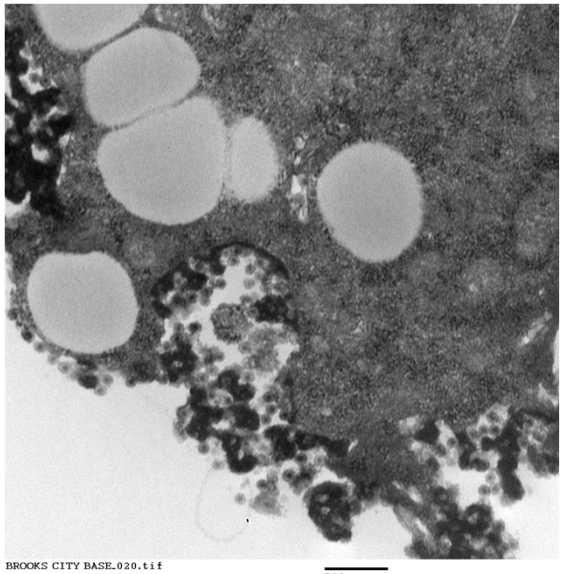
PCR products sent for sequencing:
PCR pCS20F-HpCS20R: 750pb instead of 1100pb
Nested pCS20: 280pb like *Ehrlichia ruminantium* (heartwater agent)

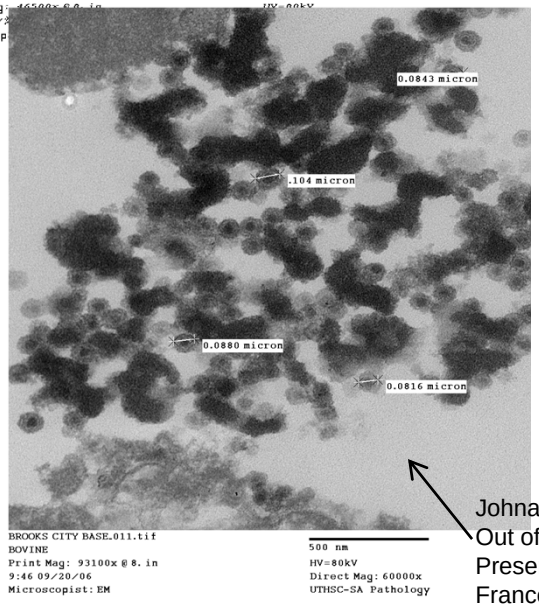
VSW and BSE ER: Viper spleen and Bovine endothelial cells infected
VSWC & BSEC: uninfected cells
T+= positive control DNA from *Ehrlichia ruminantium*
MW= ladder 100pb

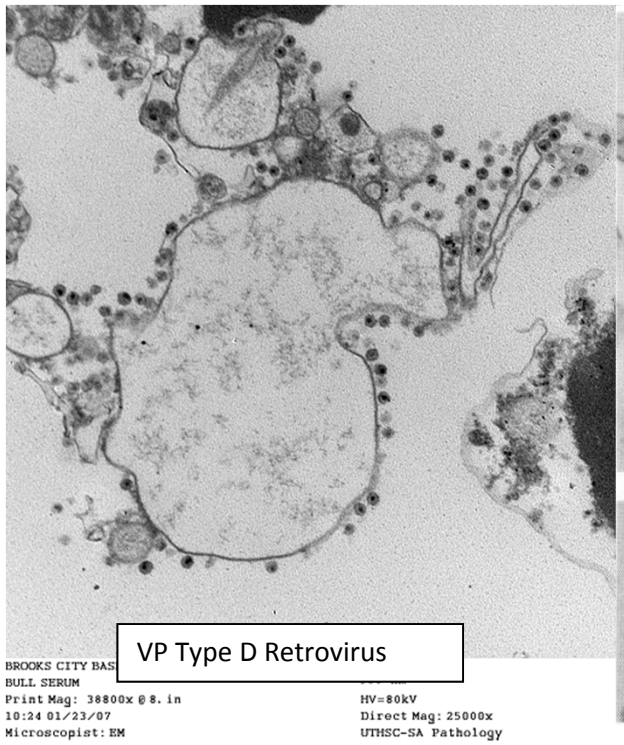
Centrifuged Bovine Endothelial Cell Supernatant Showing Rickettsia (requires many large culture flasks to accumulate this number of rickettsia)

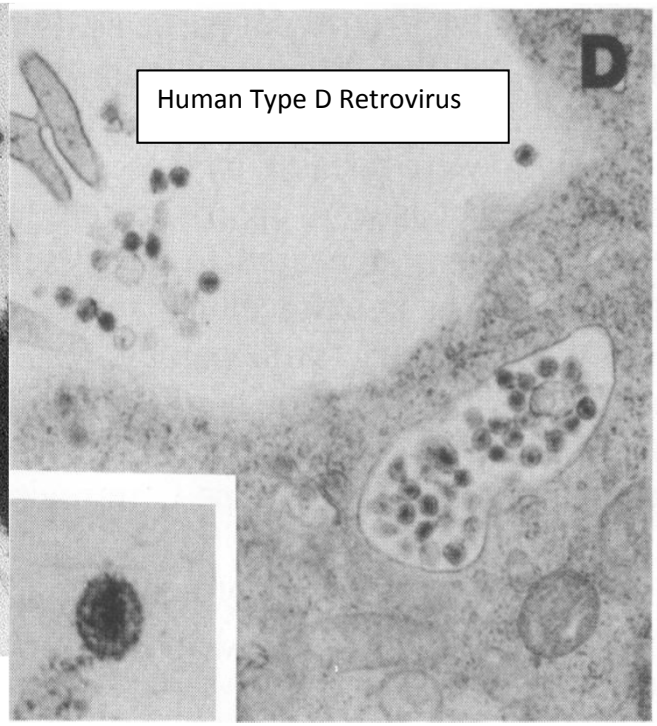


Bovine Endothelial Cells: Infected with VP Showing a Hidden Type D Immunosuppressive Retrovirus associated with the Disease and Compared to Human Type D Retrovirus







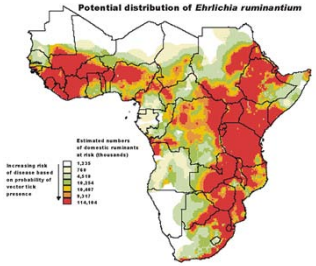


Isolation of a Type D Retrovirus from B-Cell Lymphomas of a Patient with AIDS

ROBERT C. BOHANNON,12 LAWRENCE A. DONEHOWER,1 AND RICHARD J. FORD2*
Division of Molecular Virology, Baylor College of Medicine,1 and Department of Molecular Pathology, University of Texas M. D. Anderson Cancer Center,2 Houston, Texas 77030

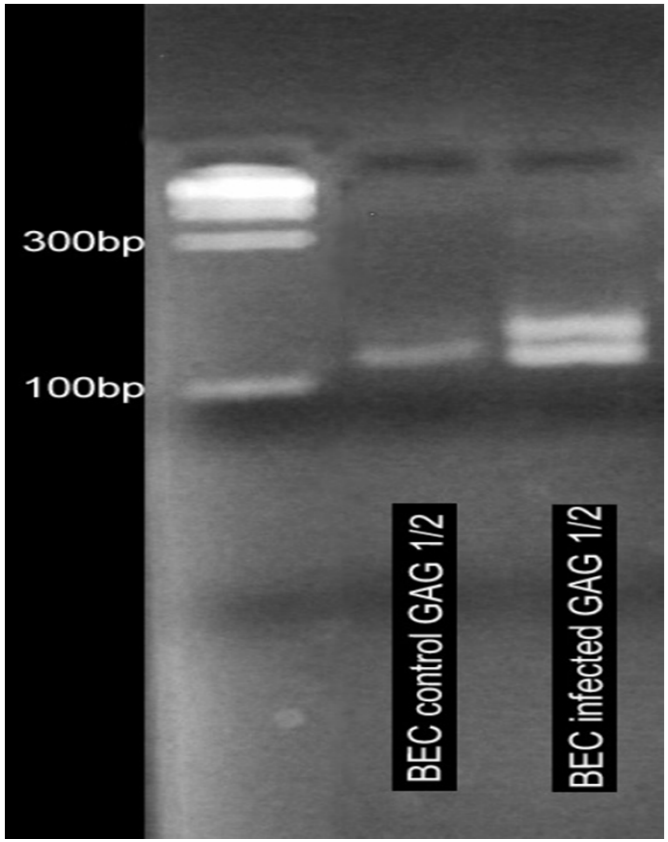
Received 29 November 1990/Accepted 23 July 1991

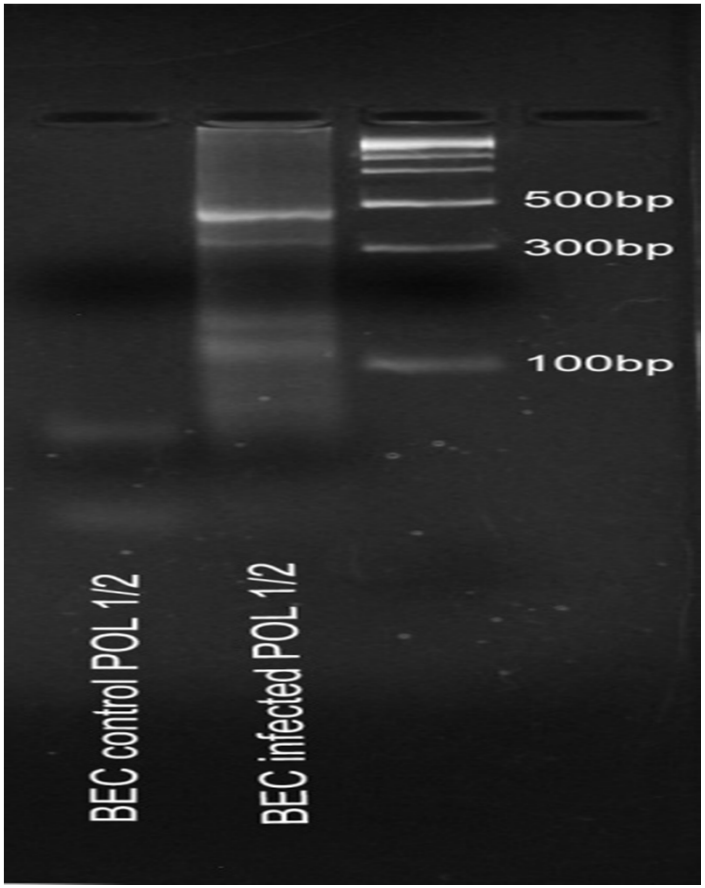
JOURNAL OF VIROLOGY, Nov. 1991, p. 5663-5672
0022-538X/91/115663-10$02.00/0



Johnathan L. Kiel, Yvette Gonzalez, Ishmael I. Rosas and David F. Vela,
Out of Africa: Do Viruses Play a Role in the Emergence of New Rickettsial Diseases?
Presentation at the 5th International Meeting on Rickettsiae and Rickettsial Diseases. Marseille, France. May 2008

New Retrovirus Infects a Wider Host Range than VSW Virus

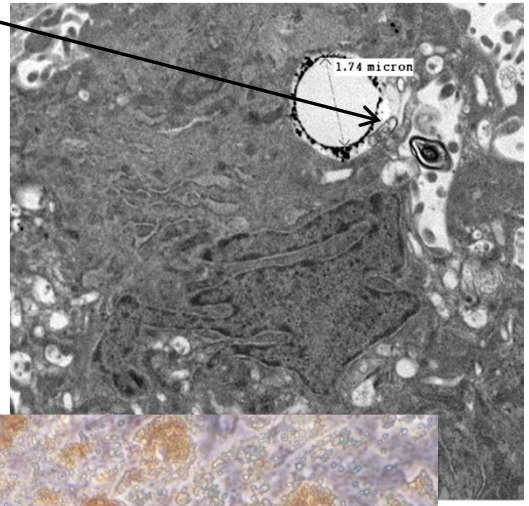




J. Kiel, Y. Gonzalez, J. Parker,C. Andrews, D. Martinez, N. Vachiery,T. Lefrancois, ANYAS 1149: 318-321 (2008)

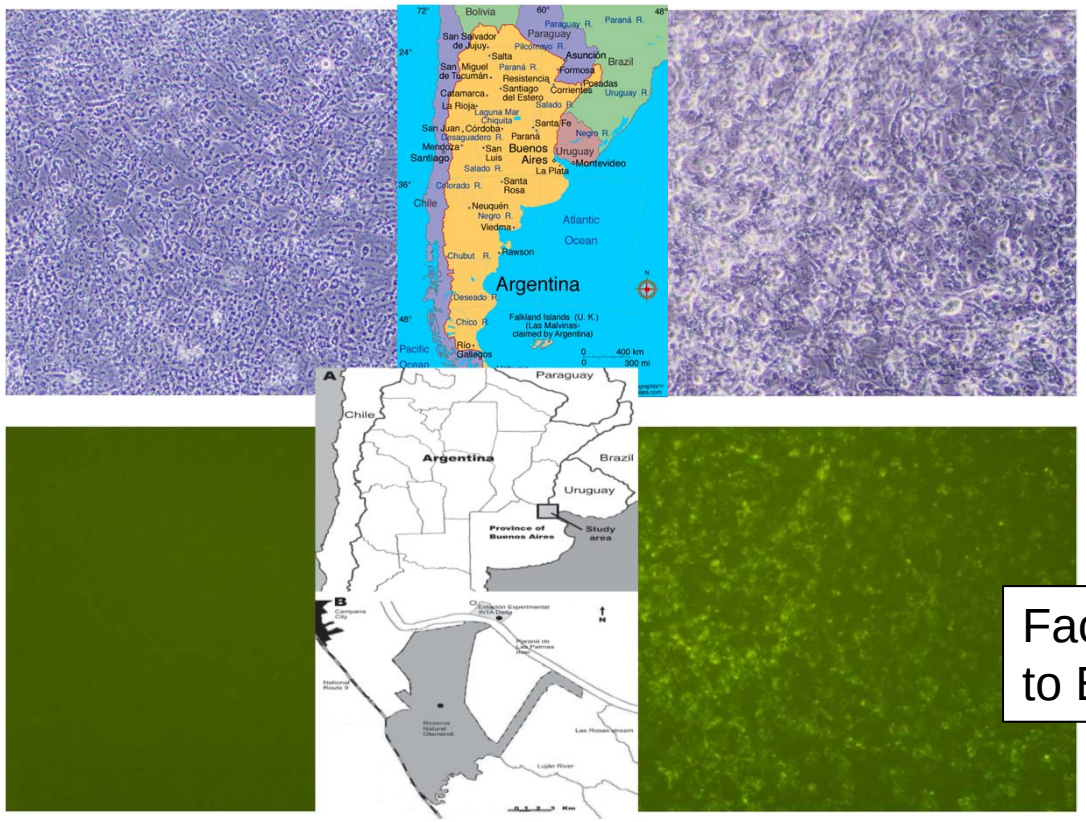
General Spotted Fever or Typhus targeting by anti-OX-19 Antigen Aptamer-coated Particles (Dr Fan by ASExpP)

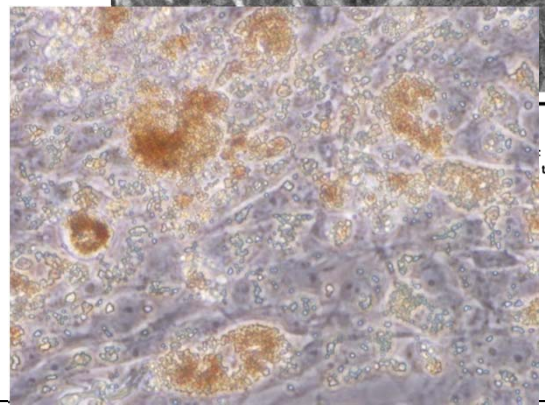
Suspected RLO attached by OX-19 aptamer to nanocrystal of iron oxide on micro mag bead



Visible and UV Light Photomicrographs after OX-19 Fluorescent Antibody Treatment

Control VH2 Cells RLO Infected VH2 Cells





Facilitated Uptake of RLO Bound to Beads (to bursting of cells)

I. Rosas and D. Vela

J. Kiel, R. Alarcon, J. Parker, J. Vivekananda,Y. Gonzalez, L. Stribling, C. Andrews, ANYAS **1081**: 434-442 (2006)
Kiel, J.L., Gonzalez, Y., Parker, J.E., Andrews, C., Martinez, D., Vacheiry, N., LeFrancois, T. , ANYAS **1149**, 318-321 (2008).

Summary

- Aptamers need to be selected under the conditions in which they are going to be used
  - **SELEX** aptamers sometime work as double-stranded contact reporting aptamers, but many times do not in spite of very low Kds
  - **ASExpP** fulfills the above criteria
- **SELEX,** by its very nature and mass action, selects for aptamers against the most abundant ligand not necessarily the most specific
  - **ASExpP**, because of its low cycle number and initial stringent conditions, selects for the highest affinity aptamer to the rarest target
- Several photochemical and electronic options exist for sensing platforms for aptamers
- Rapidity of aptamer selection in general allows for fast response to new emerging agents
- Finally, the double-stranded DNA capture elements allow for detection, identification and non-destructive safe collection for further orthogonal analysis in the lab